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COVER: Photograph of upper Boone outcrop displaying pseudo-nodular limestone bodies (gray) surrounded by tripolitic chert (white). *From: Lithologic Stratigraphic Position, Sequence and Diagenetic History, Lower Mississippian Tripolitic Chert, Northern Arkansas and Southern Missouri by S. McKim, et al., pp 165-168*

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Wave Profile for Breakdown Waves with a Large Current Behind the Wave Front

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Running Title: Wave Profile for Breakdown Waves with a Large Current Behind the Wave Front

Abstract

For analytical solution of breakdown waves with a large current behind the wave front, we employ a one-dimensional, steady-state, three-component (electrons, ions, and neutral particles) fluid model. This project involves breakdown waves propagating in the opposite direction of the electric field force on electrons, anti-force waves (return stroke in lightning); and the electron gas partial pressure is considered to provide the driving force for the propagation of the wave. The basic set of equations consists of the equation of conservation of mass flux, equation of conservation of momentum, equation of conservation of energy, plus Poisson's equation. The waves are considered to have a shock front. In this study, we examine the possibility and validity of large currents measured and reported by few investigators. Existence of a relationship between wave speed and peak current values is investigated as well.

Existence of a large current behind the wave front alters the equation of conservation of energy and Poisson's equation, as well as the shock boundary condition on electron temperature. Considering a current behind the shock front, we have made appropriate modifications in our set of electron fluid dynamical equations. Using the modified set of equations and the shock condition on electron temperature, we have been able to integrate the set of electron fluid dynamical equations for current bearing anti-force waves. For a range of wave speeds and with the largest current possible for a specific wave speed, we present the wave profile for electric field, electron velocity, and the ionization rate within the dynamical transition region of the wave for anti-force waves.

Introduction

In the late 17th to early 18th century, scientists discovered a phenomenon in which mercury gives off a glow when shaken in an evacuated glass vessel. Hauksbee (1705) was among the first to examine closely the occurrence of such luminous pulses in evacuated containers and in 1705 was able to recreate and experiment with these pulses, but focused mostly on the effects of air pressure with little regard to electrical effects. Thomson (1893) observed a moving luminous pulse in an evacuated chamber and estimated that it moved at about half the speed of light. Observations made by Beams (1930) supported this estimation. Beams explained that this phenomenon arose from the conductivity of the gas behind the pulse and that this conductivity allows the pulse to carry a potential.

In later experiments Beams, Snoddy, and Dietrich (1936) were able to find how the velocity and form of the wave varied with applied potential and air pressure. They also found that it took longer for the initial wave to propagate from the electrode to ground than for the return wave that followed to get from ground to the electrode.

Schonland (1950) made progress on determining the speed of lightning pilot streamers, though the conditions of lightning discharges differ from those in evacuated chambers. Loeb (1965) worked on corona discharge, a similar phenomenon to breakdown waves in evacuated tubes, led to further progress in understanding the propagation of such waves.

Loeb's (1965) model involves excited-state atoms emitting photons as well as the excitation of new atoms, which will in turn emit photons, continuing the process. Later this model proved not to be accurate. Observations from experiments done by Fowler and Hood (1962) with higher velocity shock waves led to a mathematical model based on fluid dynamical equations. This model led Paxton and Fowler (1962) to a theory of breakdown wave propagation in which the wave front is an electron shock wave and the partial pressure of electron gas is the primary source of motion. Their model explains wave velocity and the effects of electric fields on wave propagation in positive and negative directions.

A convention was adopted by Paxton and Fowler (1962) that separated the electron fluid dynamical waves into two different types of waves. According to this convention, if the direction of the electric field force on

electrons is in the opposite direction of wave propagation, the wave is designated to be an antiforce wave. Conversely, if the direction of the electric field force on electrons is in the same direction as the wave propagation, the wave is referred to as a proforce wave. Paxton and Fowler (1962), proposed existence of two distinct regions in breakdown waves.

The two regions of the wave are the Debye sheath layer, a thin section directly behind the shock front, where in antiforce waves, the electric field reaches a maximum but falls to a negligible value, and a thicker quasi-neutral region that comes after the Debye sheath. In this quasi-neutral region the electron gas temperature is decreased due to continued ionization while the ion and electron densities come to equilibrium.

With the two distinct categories of waves and the two regions being known, Shelton and Fowler (1968) modeled the proforce wave mathematically. This model assumes a condition of zero current behind the shock front of the breakdown wave. Fowler et al. (1984), trying to integrate the set of electron fluid-dynamical equations with the aim of meeting the physically accepted conditions at the trailing edge of the sheath region, investigated numerous approximations for the proforce wave case. This analysis led them to the conclusion that a heat conduction term must be incorporated into the conservation of energy equation. The group also concluded that there was a discontinuity in the temperature derivative at the shock front of the wave. Elastic collisions between heavy particles and electrons were also found to be resulting in a loss of energy for the electrons.

To derive their set of electron fluid-dynamical equations Shelton and Fowler (1968) considered the net current behind the shock front to be zero. This is known as the zero current condition:

$$e(N_i V - nv) = 0$$

where *e*, N_i, *V*, *n*, and *v* are the charge of an electron, ion number density inside the sheath region, wave velocity, electron number density, and electron velocity, respectively. Fowler et al. (1984) developed equations for the conservation of mass, momentum and energy coupled with Poisson's equation for the proforce wave case. These equations are:

$$\frac{d(nv)}{dx} = n\beta, \tag{1}$$

$$\frac{d}{dx}[mnv(v-V) + nkT_e] = -enE - Kmn(v-V), \tag{2}$$

$$\frac{d}{dx}[mnv(v-V)^2 + nkT_e(5v-2V) + 2envφ - \frac{5nk^2T_e}{mK}\frac{dT_e}{dx} = -3\left(\frac{m}{M}\right)nkKT_e - \left(\frac{m}{M}\right)Kmn(v-V)^2, \tag{3}$$

$$\frac{dE}{dx} = \frac{e}{\varepsilon_o}(N_i - n), \tag{4}$$

in these equations, *E, x, β, K, V, M, E_o, k* and *Φ* are the electric field and position in the wave profile, ionization frequency, elastic collision frequency, wave velocity, neutral particle mass, electric field at the wave front, Boltzmann's constant and ionization potential respectively. Also *m* and T_e are electron mass and electron gas temperature respectively. With the assumption that the net current behind of the wave front is zero, equation (4) reduces to

$$\frac{dE}{dx} = \frac{e}{\varepsilon_0} n\left(\frac{v}{V} - 1\right). \tag{5}$$

Fowler et al. (1984) applied a set of non-dimensional variables to the set of electron fluid dynamical equations to reduce the set to non-dimensional form. The variables are:

$$\omega = \frac{2m}{M}, \quad \kappa = \frac{mVK}{eE_o}, \quad \mu = \frac{\beta}{K},$$
$$\alpha = \frac{2e\phi}{mV^2}, \quad \psi = \frac{v}{V}, \quad \nu = \frac{2e\phi n}{\varepsilon_o E_o^2},$$
$$\theta = \frac{T_e k}{2e\phi}, \quad \eta = \frac{E}{E_o}, \quad \xi = \frac{xeE_o}{mV^2}$$

Where, the dimensionless variables *ν, ψ, θ, μ, η* and *ξ* are defined as electron number density, electron velocity, electron temperature, ionization rate, net electric field, and position inside the sheath region of the wave, respectively. *α* and *κ* represent wave parameters. Therefore, in dimensionless form, the complete set of equations for the proforce case are

$$\frac{d}{d\xi}[\nu\psi] = \kappa\mu\nu, \tag{6}$$

$$\frac{d}{d\xi}[\nu\psi(\psi-1) + \alpha\nu\theta] = -\nu\eta - \kappa\nu(\psi-1), \tag{7}$$

$$\frac{d}{d\xi}\left[\nu\psi(\psi-1)^2+\alpha\nu\theta(5\psi-2)+\alpha\nu\psi+\frac{5\alpha^2\nu\theta}{\kappa}\frac{d\theta}{d\xi}\right]=-\omega\kappa\nu[3\alpha\theta+(\psi-1)^2] \quad (8)$$

$$\frac{d\eta}{d\xi}=\frac{\nu}{\alpha}(\psi-1). \quad (9)$$

With the proforce wave case equations completed and solved, attention shifted to the antiforce case. However, there were many problems in formulating a set of equations for antiforce waves similar to the set of equations describing proforce waves. To apply the set of electron fluid-dynamical equations to antiforce waves, modification of the equations is required. Additional changes must be considered and modifications must be made to the non-dimensional variables used in the proforce case in order for application to the antiforce case to be accurate.

In order for the set of electron fluid dynamical (EFD) equations to be non-dimensional, the following dimensionless variables were derived by Hemmati (1999) to the EFD equations (1-3, 5):

$$\omega=\frac{2m}{M},\ \kappa=-\frac{mVK}{eE_o},\ \mu=\frac{\beta}{K},$$
$$\alpha=\frac{2e\phi}{mV^2},\ \psi=\frac{\nu}{V},\ \nu=\frac{2e\phi n}{\varepsilon_o E_o^2},$$
$$\theta=\frac{T_e k}{2e\phi},\ \eta=\frac{E}{E_o},\ \xi=-\frac{xeE_o}{mV^2}$$

It was previously assumed by Sanmann and Fowler (1975) that μ, the ionization rate, was purely a function of θ, electron temperature. Fowler et al. (1984) concluded that this was not the case. In fact, calculating the ionization rate within the sheath region of the wave, random and directed electron motions must be taken into account. Shelton assumed that μ was constant and it would later be determined by Fowler et al. (1984) that the ionization rate does indeed remain substantially constant near the front of the sheath region, though it changes later. It was thought by Shelton that heat conduction was negligible in the sheath region and throughout the quasi-neutral region. It was determined by Fowler et al. (1984) that this was an error in the formulation of the equations and a term for heat conduction was included in the equations.

In the laboratory frame, ion motion is considered negligible due to the fact that no Doppler shift has been observed in the analysis of radiation emitted from the propagation of breakdown waves. In the wave frame, heavy particles will be moving in the negative x direction. Therefore, heavy particle speed, *V*, is negative, while E_0 is positive, κ and ξ are therefore both negative.

After applying these dimensionless variables, the EFD equations (1-3, 5) become the non-dimensional set of equations describing the antiforce wave case. The following equations are the complete set of non-dimensional EFD equations developed by Hemmati (1999) for the antiforce wave:

$$\frac{d}{d\xi}[\nu\psi]=\kappa\mu\nu, \quad (10)$$

$$\frac{d}{d\xi}[\nu\psi(\psi-1)+\alpha\nu\theta]=\nu\eta-\kappa\nu(\psi-1), \quad (11)$$

$$\frac{d}{d\xi}\left[\nu\psi(\psi-1)^2+\alpha\nu\theta(5\psi-2)+\alpha\nu\psi-\frac{5\alpha^2\nu\theta}{\kappa}\frac{d\theta}{d\xi}\right]=2\nu\eta(\psi-1)-\omega\kappa\nu[3\alpha\theta+(\psi-1)^2], \quad (12)$$

$$\frac{d\eta}{d\xi}=-\frac{\nu}{\alpha}(\psi-1). \quad (13)$$

Letting I_1 represent the current behind the wave front we get

$$I_1=eN_iV_i-env. \quad (14)$$

Solving for N_i gives

$$N_i=\frac{I_1}{eV}+\frac{nv}{V}. \quad (15)$$

Substituting this in equation (4) results in

$$\frac{dE}{dx}=\frac{e}{\varepsilon_0}\left(\frac{I_1}{eV}+\frac{nv}{V}-n\right). \quad (16)$$

Substituting the dimensionless variables in previous equation results in

$$\frac{d\eta}{d\xi}=\frac{\kappa I_1}{\varepsilon_0 KE_0}-\frac{\nu}{\alpha}(\psi-1). \quad (17)$$

Finally, letting ι be the dimensionless current representation of $\frac{I_1}{\varepsilon_0 KE_0}$ gives

$$\frac{d\eta}{d\xi}=\kappa\iota-\frac{\nu}{\alpha}(\psi-1). \quad (18)$$

Equation (18) can be solved for $\nu(\psi - 1)$ and the result can be substituted into equation (12) giving the final form of the conservation of energy equation.

The preceding equations derived by Hemmati et al (2011) give us our final form of the EFD equations for antiforce waves:

$$\frac{d}{d\xi}[\nu\psi] = \kappa\mu\nu, \tag{19}$$

$$\frac{d}{d\xi}[\nu\psi(\psi - 1) + \alpha\nu\theta] = \nu\eta - \kappa\nu(\psi - 1), \tag{20}$$

$$\frac{d}{d\xi}\left[\nu\psi(\psi - 1)^2 + \alpha\nu\theta(5\psi - 2) + \alpha\nu\psi - \frac{5\alpha^2\nu\theta}{\kappa}\frac{d\theta}{d\xi} + \alpha\eta^2\right] = 2\eta\kappa\iota\alpha - \omega\kappa\nu[3\alpha\theta + (\psi - 1)^2], \tag{21}$$

$$\frac{d\eta}{d\xi} = \kappa\iota - \frac{\nu}{\alpha}(\psi - 1). \tag{22}$$

All quantities in this set of equations are intrinsically positive, including, κ. Equations (19-22) describe the final set of EFD equations with a large current behind the wave front for antiforce waves.

It was assumed by Shelton and Fowler (1968) that ionization rate was constant, and then later thought by Sanmann and Fowler (1975) to be a function of electron temperature only. A study by Fowler (1983) showed that in the calculation of ionization rate, ionization from both random and directed electron motion must be considered. Therefore, we have used an expression derived by Fowler (1983) to calculate the ionization rate within the sheath region of the wave that takes into account ionization from both directed and random electron motions. Thus,

$$\mu = \mu_0 \int_{\frac{1}{\sqrt{2\theta}}}^{\infty} \sigma_i z^2 dz \int_B^{\infty} \frac{e^{-(z-u)^2} - e^{-(z+u)^2}}{u} du\, e^{-2Cu}$$

where $B = (1 - \psi)/\sqrt{2\alpha\theta}$ and $C = \kappa\sqrt{2\alpha\theta}/\eta$.

Results and Discussion

Uman et al. (2000) reported return stroke wave speeds as low as 0.46 X 10^8 m/s. Similarly, Rakov (2000) in his study of positive and bipolar lightning discharges measured a range of wave-speeds in agreement with other experimental works. His reported wave speed values were between $0.3X10^8$ m/s – $1.7X10^8$ m/s. Rakov (2000) also reported in the study of the characteristics of positive and bipolar lightning that the return stoke current ranged from 10 kA – 40 kA depending upon experimental location. While studying rocket triggered lightning strokes, Wang et al. (1999) observed a peak current value of around 12 kA – 21 kA. During the investigation of the time derivative of the electric field in triggered lightning strokes, Uman et al. (2000) observed current values for return stokes as large as 30.4 kA.

For lightning return strokes, the current values generally reported by investigators lie within the range of 10-40 kA. However, few investigators report existence of currents as high as 300 kA (Rakov, 2000).

A trial and error method was used to integrate equations (19 – 22) through the sheath region of the wave. The largest current, ι, that led to successful solutions was chosen for given wave speeds, α, and values for the wave constant, κ, electron velocity, ψ, and electron number density, ν, were chosen so that integration of the set of equations led to a conclusion consistent with the expected conditions at the trailing edge of the sheath. This was done by repeatedly adjusting κ, ψ, and ν until integration led to results that were in agreement with the expected conditions at the end of the dynamical transition region of the wave.

Certain boundary conditions must be met in order for integration to be successful. Namely, η_2, the electric field at the end of the sheath region, must approach 0 and, ψ_2, the dimensionless electron velocity at the end of the sheath region, must approach 1.

The following initial variable values lead to successful integration of the set of electron fluid dynamical equations and were found to satisfy the boundary conditions at the end of the sheath region of the wave:

α = 0.001, ι = 7, κ = 0.144, ψ_1 = 0.4721, ν_1 = 0.2161

α = 0.01, ι = 5, κ = 0.13, ψ_1 = 0.7, ν_1 = 0.7696

α = 0.1, ι = 1, κ = 0.44, ψ_1 = 0.8321, ν_1 = 0.71

α = 1, ι = 0.25, κ = 0.18, ψ_1 = 0.75, ν_1 = 0.7

In figure 1 the electric field intensity, η, is shown as a function of the electron velocity, ψ. The gaps in the curves α = 0.001 and α = 0.01 are due to the fact that only one out of ten data points calculated were plotted. In this figure we can clearly see that the electric field is falling to 0 and the electron velocity is approaching 1, which satisfies the conditions at the trailing edge of the wave.

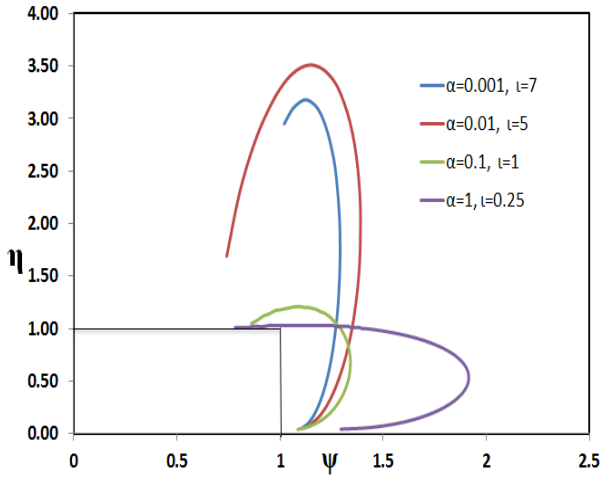

Figure 1: Dimensionless electric field, η, as a function of dimensionless electron velocity, ψ, within the sheath region of the wave.

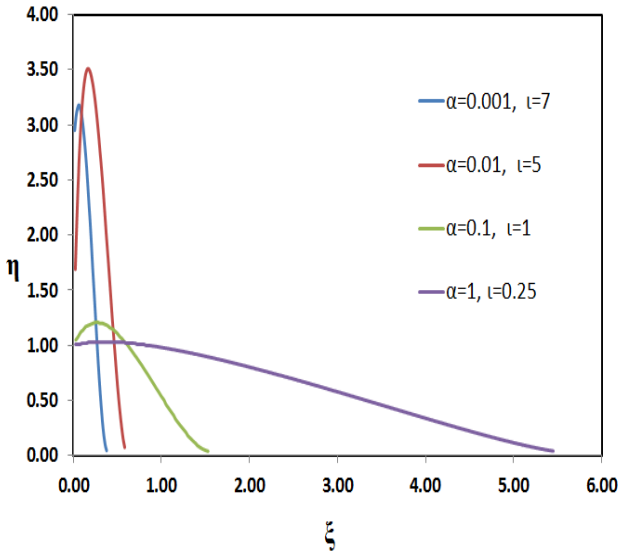

Figure 2: Dimensionless electric field, η, as a function of position, ξ, within the sheath region of the wave.

In figure 2 the electric field intensity, η, is shown as a function of the position, ξ, within the sheath region of the wave. The fall of the electric field to zero marks the end of the sheath region of the wave. Sanmann and Fowler (1975) applied fluid dynamic techniques to antiforce waves and for a wave speed of 10^7 m/s found a total sheath thickness of 0.5 m. Fujita et al. (2003), in measuring electron densities behind shock waves, reported a sheath thickness of 0.05 m. Our data for ξ for waves at speeds of 3 X 10^7 m/s show a sheath thickness of 0.025 m.

In figure 3, the dimensionless ionization rate, μ, is shown as a function of the dimensionless position, ξ, within the sheath region of the wave. To reduce the computation time, ionization rate was kept constant for ten integration steps and calculated every tenth step. To keep track of variable changes while integration and computation occur, only every tenth integration step is printed so that all previous data lines can be displayed simultaneously on the computer screen. Therefore, regarding change in ionization rate, every hundredth integration step is displayed. The sharp changes in the graphs are an unavoidable consequence of keeping the ionization rate constant and displaying the change in ionization rate only every hundredth step. Shelton and Fowler (1968) assumed that the ionization rate would remain constant through the sheath region of the wave. We see here that ionization rate remains constant for a short time behind the wave front, but generally changes as we move through the sheath region of the wave.

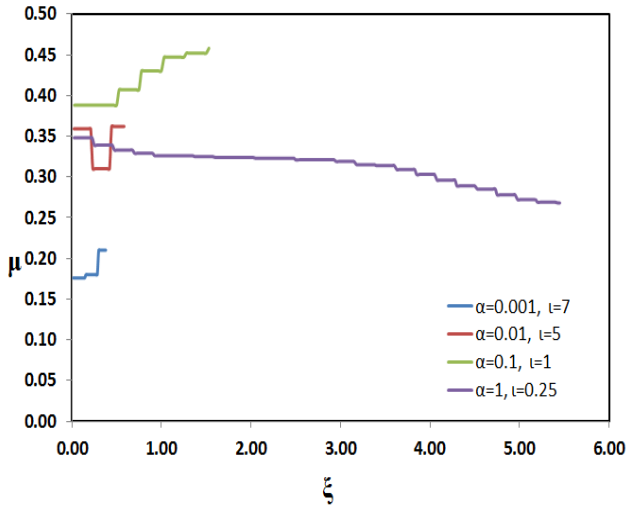

Figure 3: Dimensionless ionization rate, μ, as a function of position, ξ, within the sheath region of the wave.

For return lightning strokes, some investigators have suggested the existence of a relationship between the peak current values and wave speed values (Wagner 1963); however, some others, (Willett et al. 1989), especially researchers investigating triggered lightning in Florida, disagree with the existence of such a relationship. For lightning return strokes, our solutions indicate, as the wave speed increases, the current values that it can support increases as well.

Conclusions

We have considered the existence of a large current behind the wave front and found a range of wave speeds and their corresponding maximum current values for

which integration of the electron fluid-dynamical equations led to results in agreement with the boundary conditions at the trailing edge of the sheath region. For lightning return strokes, our solutions also confirm the existence of large currents. Agreement between the results of the solutions of the electron fluid-dynamical equations with experimental evidence such as wave velocity and electron number density are conformations of the validity of the fluid model.

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Vertebrate Natural History Notes from Arkansas, 2017

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Running Title: Vertebrate Natural History Notes, 2017

Abstract

Because meaningful observations of natural history are not always part of larger studies, important pieces of information often are unreported. Small details, however, can fills gaps in understanding and also lead to interesting questions about ecological relationships or environmental change. We have compiled recent observations of foods, reproduction, record size, parasites, and distribution of 30 species of fishes, new records of distribution and parasites of 2 species of amphibians, and new records of distribution, parasites, reproduction and anomalies of 11 species of mammals.

Introduction

Human alteration of environments and introduction of non-native species constantly alters relationships and life history parameters of species studied by vertebrate field biologists. Distribution and natural history of many species within Arkansas is becoming better documented, but much remains to be discovered and reported. We have developed a series of articles to update the state of knowledge of the natural history of Arkansas's vertebrates (see Tumlison 2016 and references therein). Herein, we include previously unreported records of distribution, parasites, reproduction, food habits, disease, and other aspects of natural history of the vertebrates of Arkansas. Voucher specimens are deposited in the vertebrate collections at Henderson State University (HSU).

Methods

Some fishes were collected by use of 3.1 × 1.8 m or 6.1 × 1.8 m seines with 3.2 mm mesh, or by use of a backpack electroshocker. Goldeyes were collected by use of the Missouri Trawl (Herzog and Hrabik 2012), which is designed to skim the bottom of streams and rivers where no other gear can be effectively deployed. Specimens were preserved in 10% formalin and stored in 45% v/v isopropanol, or photographic vouchers were taken. Localities are reported as GPS (latitude and longitude) coordinates when available. Vouchers of parasites were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln.

Results and Discussion

CLASS ACTINOPTERYGII

Hiodontidae – Mooneyes and Goldeyes

***Hiodon alosoides* (Rafinesque) – Goldeye.** The diet of the Goldeye is variable over its range (Robison and Buchanan 1988). In South Dakota, fish and both terrestrial and aquatic insects dominate the diet (Johnson 1963). No reports of foods consumed by *H. alosoides* are available in Arkansas. On 16 October 2015, 3 Goldeyes (115–125 mm TL) were collected from the Mississippi River at Sans Souci Landing S of Osceola, Mississippi Co. (35.655432°N, 89.926073°W) which had eaten Cottonwood Leaf Beetles, *Chrysomela scripta* Fabricius (Fig. 1). This marks the first report of Goldeye

feeding on terrestrial coleopterans in Arkansas. This fish is listed as a Species of Greatest Conservation Need in Arkansas (Anonymous 2016).

Figure 1. Several elytra of cottonwood leaf beetles removed from stomachs of *Hiodon alosoides*.

***Cyprinidae* – minnows and carps**

***Campostoma spadiceum* (Girard) – Highland Stoneroller.** This fish is the most recently described stoneroller species in Arkansas and little is known of its biology, particularly reproduction. Cashner et al. (2010) observed that nuptial colors of adult males peaked in March or April. W. J. Matthews (*pers. comm*.) commented that tubercled males were taken in March and up to 25 April, thus indicating a typical spring spawning season. Quite unexpectedly, on 15 November 2015, a tuberculate male (112 mm TL) was collected from Ten Mile Creek near Lonsdale, Saline Co. (34.545274°N, 92.753888°W) which is long after the typical breeding season. A 91 mm TL female with egg sacs was collected on 11 April 2015 from Wingfield Creek at AR St. Hwy 8, Clark Co. (34.187906°N, 93.255394°W). A tubercled male and gravid female were collected from Mill Creek on the campus of Henderson State University, Arkadelphia, AR, on 3 March 2017 (34.133041°N, 93.059936°W) indicating an early start of the breeding season.

***Luxilus chrysocephalus* Rafinesque – Striped Shiner.** Robison and Buchanan (1988) commented that spawning in Arkansas occurred from late spring to early summer. Several recent observations add to the scant information about the reproductive period of this cyprinid in Arkansas. On 10 June 2016, a 118 mm TL tubercled male in full breeding coloration (photographed by CTM) was captured in Butcherknife Creek, Polk Co. (34.468688°N, 93.992288°W). On 23 May and 28 May 2016, a 99 mm TL male and 4 females (90, 93, 93, and 101 mm TL) with eggs were collected from Abernathy Spring, Polk Co. (34.468108°N, 93.947656°W). Additional females with eggs were taken on 1 May 2015 (90 mm TL female) from Garland Co., Bear Creek (34.534915°N, 93.286449°W) and 2 females (95 mm TL), were captured on 21 May 2016 in Clark Co., Wingfield Creek at AR St. Hwy 8 (34.187906°N, 93.255394°W).

***Notropis atherinoides* Rafinesque – Emerald Shiner.** Little life history information is available on southern populations of *N. atherinoides* and little is known on Arkansas populations. Breeding is believed to occur in Arkansas during late spring and early summer (Robison and Buchanan 1988). On 22 April 2016, a 68 mm TL female with 2 large egg sacs was collected at the Calion Spillway below Calion Lake, Union Co. (33.325312°N, 92.526721°W).

***Luxilus zonatus* (Agassiz) – Bleeding Shiner.** Breeding of this shiner in Arkansas is believed to occur from late April to late June (Robison and Buchanan 1988). Our collection of a ripe female (105 mm TL) with eggs on 8 July 2015 from North Big Creek at AR St. Hwy 354, Sharp Co. (36.157629°N, 91.514177°W) lengthens the known spawning period into early July.

Lythrurus* cf. *umbratilus. This undescribed form from the upper Ouachita River (see Robison and Buchanan 1988) currently is being studied by HWR and W. C. Starnes. The following information is the first concerning the reproductive period of this Arkansas endemic form. Breeding individuals were collected in June and July as follows: on 8 June 2015, a 58 mm TL male in full breeding coloration was taken from Bear Creek at Bear, Garland Co. (34.534915°N, 93.286449°W). On 2 July 2014, a 63 mm TL male in breeding color and a 49 mm TL female with eggs was collected from the same site. On 21 July 2014, another 82 mm TL female with ova was taken from the same site. On 21 June 2013, a male with nuptial tubercles and breeding color was photographed from Walnut Creek at Camp Clearfork, off US Hwy 270, Garland Co. (34.507°N, 93.395°W).

***Lythrurus umbratilus* (Girard) – Redfin Shiner.** Excepting the undescribed form, Redfin Shiners occur statewide except in the upper White River (Robison and Buchanan 1988). Spawning of *L. umbratalis* in Arkansas appears to be extended from late April to August (Robison and Buchanan 1988). However, variation could exist within subspecies, and the following information refers specifically to *Lythrurus umbratilis cyanocephalus* (Copeland), the Northern

Redfin Shiner, about which nothing is known in Arkansas. On 5 July 2014, a 66 mm TL female *L. u. cyanocephalus* with eggs was collected from the Rolling Fork River off Johnson Bridge Road in Sevier Co., just W of DeQueen (34.064539°N, 94.380613°W).

***Semotilus atromaculatus* (Mitchill) – Creek Chub**. The maximum length for Creek Chubs was given by Trautman (1957) as 303 mm (11.9 in.). This was from Ohio, but no maximum length has been recorded for Arkansas. On 19 February 2017, Rana Tumlison caught a specimen on rod and reel from Spring Creek on the E side of Lake Springdale, Benton Co., AR, that measured 295 mm (11.5 in.). This large individual sets our largest known specimen from Arkansas.

***Catostomidae* - Suckers**

***Moxostoma poecilurum* Jordan – Blacktail Redhorse.** Spawning in Arkansas typically occurs from late April through May (Robison and Buchanan 1988), thus the discovery of a 242 mm TL female with eggs taken on 12 October 2015 from West Tulip Creek, Dallas Co. (33.906488°N, 92.730825°W) was unexpected.

***Moxostoma duquesnei* (Lesueur) – Black Redhorse.** Spawning of this redhorse sucker in Missouri takes place in late April or early May (Pflieger 1997); however, no information currently exists for Arkansas populations. On 5 April 2016, a 382 mm TL female with eggs weighing 75.5 g was taken from below the dam on the White River at Batesville, Independence Co. (35.755847°N, 91.638138°W).

***Ictaluridae* – Catfishes**

***Noturus exilis* Nelson – Slender Madtom.** Spawning of *N. exilis* occurs May through July in Illinois (Mayden and Burr 1981) and from late April to early June in Oklahoma (Vives 1987). Robison and Buchanan (1988) collected ripe females during late April and May in Arkansas. We report ripe females of *N. exilis* in May and July from the state. On 15 May 2015, an 88 mm TL female with eggs was collected from Flint Creek off AR St. Hwy 59 at Gentry, Benton Co. (36.242716°N, 94.487408°W) and 3 females (77, 91, 96 mm TL) with eggs were taken on 5 July 2015 from the North Fork of White Oak Creek at AR St. Hwy 23, N of Ozark, Franklin Co. (35.55574°N, 93.86210°W), and a 102 mm TL female with eggs was collected on 5 July 2015 in Fane Creek off Forest Service Road 1520 at Deepwoods Trail, Franklin Co. (35.69635°N, 93.82716°W).

***Noturus gyrinus* Mitchill – Tadpole Madtom.** This madtom spawns in June or July in Missouri (Pflieger 1997); however, little is known about Arkansas spawning times except that small young have been found in early July (Robison and Buchanan 1988). It appears that the breeding season may begin somewhat earlier in Arkansas. On 11 April 2015, a 61 mm TL female with eggs was collected from an unnamed pond at 34.11700°N, 93.0073°W, on AR St. Hwy 51, 3.6 km E of Arkadelphia, Clark Co.

***Esoscidae* – Pickerel**

***Esox americanus* Gmelin – Redfin Pickerel.** In Canada, Crossman (1962) reported that *E. americanus* usually feeds on fishes and only occasionally on aquatic insects and crayfish. In Lake Ouachita, Garland Co., AR, this predator mostly consumed fishes, but also took freshwater shrimp (*Palaemonetes kadiakensis*) commonly (Tumlison et al. 2007). On 10 July 2016, 2 individuals (109, 143 mm TL) collected in Locust Bayou at US Hwy 278NE, Calhoun Co. (33.557459°N, 92.675849°W), were found to have eaten *Orconectes* crayfishes of the subgenus *Pennides*.

***Apherododeridae* – Pirate Perch**

***Apherododerus sayanus* Gilliams – Pirate Perch.** No reports of foods eaten by *A. sayanus* in Arkansas are available; however, Forbes and Richardson (1920) in Illinois and Flemer and Woolcott (1966) in Virginia reported this species feeds primarily on insects. In North Carolina, Shepherd and Huish (1978) reported a diet of Cladocera, dipteran larvae, isopods, and amphipods. We found 2 specimens of *A. sayanus* (83, 90 mm TL) to have fed on scuds (*Hyalella azteca*) on 23 April 2016 at Spring Mill (Big Spring) off AR St. Hwy 69, S of Cushman, Independence Co. (35.828214°N, 91.724288°W).

In Arkansas, this species spawns in May and early June (Robison and Buchanan 1988). On the same date and site above, we collected 2 male *A. sayanus* (55-58 mm TL) full of sperm which appears to push back the date of spawning into late April in the state.

***Fundulidae* – Topminows**

***Fundulus blairae* Wiley and Hall – Western Starhead Topminnow.** Little is known of the biology of this fundulid (Robison and Buchanan 1988). On 9 July 2016, a 37 mm TL female with eggs was collected 8 km W of Horatio off AR St. Hwy 24, at a private pond near the Rolling Fork backwater, Sevier Co. (33.954089°N, 94.427058°W).

This fish is listed as a Species of Greatest Conservation Need in Arkansas (Anonymous 2016), but no information regarding food habits is known for *F.*

blairae. Examination of foods of *F. blairae* (32-41 mm TL) from this same site, collected on 27 August and 4 September 2016, revealed that 6 of 37 (16%) consumed unidentified seeds (Fig. 2).

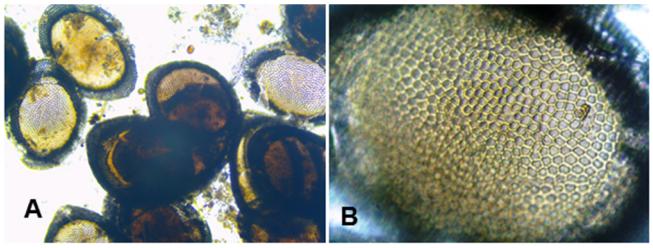

Figure 2. Unidentified seeds found in the stomach of *Fundulus blairae*. A. Several seeds. B. Close-up of single seed.

***Fundulus catenatus* (Storer) – Northern Studfish.** Surprisingly, little is known about the biology of this common and widespread topminnow in Arkansas (Robison and Buchanan 1988). Rice (1942) reported it was a surface feeder eating primarily insects and small crustaceans; however, nothing in Arkansas has been published on feeding habits. On 22 November 2016, a 73 mm TL *F. catenatus* was taken from Ten Mile Creek off US Hwy 70 near Lonsdale, Saline Co. (34.545274°N, 92.753888°W) and found to have a small species of cicada in its stomach contents, which marks the first report of this organism being eaten by this species. Earlier, on 21 April 2016, 2 ants were found in the gut of a *F. catenatus* taken from the Caddo River off Manford Road at Caddo Gap, Montgomery Co. (34.399855°N, 93.621693°W).

Robison and Buchanan (1988) reported this topminnow had a protracted spawning period breeding from May through August, although nothing specific is known about its reproductive biology in Arkansas. On 1 May 2015, a 75 mm TL female with eggs was taken from Walnut Creek off Hickorynut Mountain Road, Garland Co. (34.533903°N, 93.371055°W), and a 65 mm TL female with eggs was found in nearby Bear Creek, Garland Co. (34.534915°N, 93.286449°W).

***Fundulus chrysotus* (Gunther) – Golden Topminnow.** Very little is known regarding the reproductive biology of this killifish (Robison and Buchanan 1988), rendering the following observations important to understanding the biology of this species in Arkansas. On 22 April 2016, we found 3 females (42-50 mm TL) with mature eggs below the Calion Spillway at Calion Lake, Union Co. (33.325312°N, 92.526721°W). On 11 July 2016, we collected a 50 mm TL female with eggs which was being pursued by several adult males (65-82 mm TL) in full breeding coloration at Cane Creek Lake at Cane Creek State Park, E of Star City, Lincoln Co. (33.916812°N, 91.765855°W).

***Fundulus dispar* (Agassiz) – Starhead Topminnow.** While spawning of *F. dispar* occurs in late spring to early summer (Robison and Buchanan 1988), nothing specific is known of its reproductive biology in Arkansas. On 22 April 2016 below the Calion Spillway at Calion Lake, Union Co. (33.325312°N, 92.526721°W), we collected a 44 mm TL female containing eggs.

***Cottidae* – Sculpins**

***Cottus carolinae* (Gill) – Banded Sculpin.** Cooper (1975) reported foods consumed by *C. carolinae* in North Fork River in Missouri. She found crayfish were the most important food item, whereas in northeastern Oklahoma, Tumlison and Cline (2002) found other small aquatic invertebrates to dominate the diet. Herein we report a male *C. carolinae* (126 mm TL) collected on 17 November 2012 from Flint Creek off Fairmount Road at Springtown, Benton Co. (36.252632°N, 94.440359°W) which had a midget crayfish (*Orconectes nana*) in its gut. This crayfish is listed as a Species of Greatest Conservation Need in Arkansas (Anonymous 2016). Tumlison and Cline (2002) also found sculpins to consume the Oklahoma salamander (*Eurycea tynerensis*), another species of conservation concern.

Adults in breeding condition have been taken from the White River in Arkansas in mid-February (Robison and Buchanan 1988). We collected a 118 mm TL female containing mature eggs much later, on 17 November 2015, at the same Flint Creek locality listed previously.

***Centrarchidae* – Sunfishes**

***Lepomis cyanellus* Rafinesque – Green Sunfish.** On 13 October 2016, 2 adult *L. cyanellus* were collected from Pickles Gap Creek, Faulkner Co. (35.12551°N, 92.400955°W), that were noticeably emaciated and possessed unknown white growths on their bodies, particularly their dorsal fins (Fig. 3). Subsequent examination of these growths revealed the ciliate *Epistylis* sp. McAllister et al. (2016*c*) recently reported an *Epistylis* sp. from *L. cyanellus* from Ten Mile Creek, Saline Co. We document a second occurrence of this ciliate in green sunfishes, and add a new drainage, the Arkansas River, to its distribution on fishes in the state.

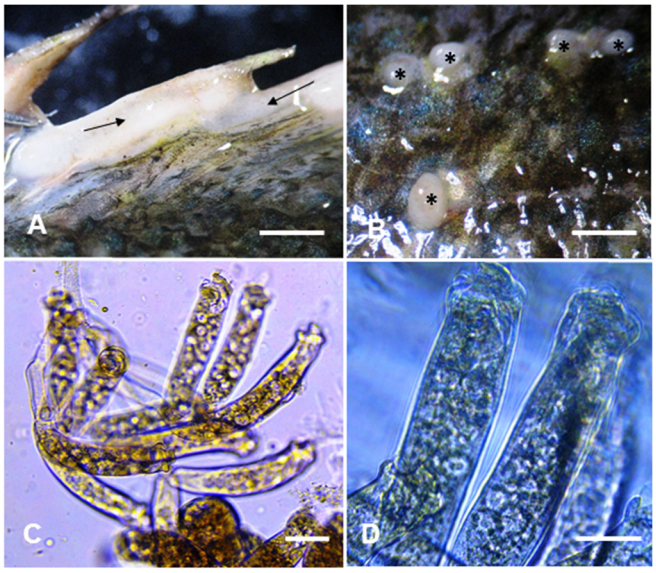

Figure 3. *Epistylus* sp. on *Lepomis cyanellus*. A. Whitish growth (arrows) on dorsal fin between spines. B. Pustule-like growths (asterisks) on side of fish. C. Colonies showing group of zooids, unstained. D. Close-up of 2 elongate zooids, unstained. Scale bars A-B = 2 mm, C-D = 100 µm.

***Percidae* – Darters**

***Etheostoma chlorosoma* (Hay) – Bluntnose Darter.** In Texas, spawning occurs from early January to late March (Hubbs 1985). Reproductive season in Arkansas has not been studied, but is believed to be in the spring. On 11 April 2015, 2 females (48, 52 mm TL) with eggs were taken from Saline Bayou off AR St. Hwy 51 in Clark Co. (34.11654°N, 93.030523°W).

In addition, 5 of 5 (100%) *E. chlorosoma* (47-51 mm TL) collected on 11 April 2015 from an unnamed pond in Clark Co. (34.1170°N, 93.0073°W) possessed the monogenean, *Aethycteron chlorosomus* (Harrises and Vickery, 1970) on their gills (HWML 139318). Mean intensity was 14.4 ± 2.6 (range = 12-18) worms. This parasite was previously known from *E. chlorosoma* and Speckled Darter (*E. stigmaeum*) from Mississippi (Harrises and Vickery 1970). We document a new state record for *A. chlorosomus*, the first time the parasite has been reported from west of the Mississippi River, and the third time the genus *Aethycteron* has been reported from an Arkansas fish (McAllister et al. 2016*a*; Cloutman and McAllister 2017).

***Etheostoma collettei* Birdsong and Knapp – Creole Darter.** Little is known about the biology of this darter (Robison and Buchanan 1988), but HWR (*unpubl. obs.*) has found it feeds mainly on aquatic insects. However, a new category of food items is herein added to its food habits as on 22 November 2016 at Ten Mile Creek off US Hwy 70 near Lonsdale, Saline Co. (34.545274°N, 92.753888°W), 2 individuals (52, 58 mm TL) were found with aquatic mites in their gut, marking the first time this food item has been recorded for this species.

***Etheostoma euzonum* (Hubbs and Black) – Arkansas Saddled Darter.** Little is known of the life history of this species (Robison and Buchanan 1988); however, the spawning season in Arkansas extends at least from late March through May (Hubbs 1985). A large male in breeding coloration was captured on 24 April 2016 from the Middle Fork of the Little Red River just W of Shirley, Van Buren Co. (35.651965°N, 92.320282°W). Robison and Buchanan (1988) only showed 2 localities for this darter in the Little Red River system, thus it is rare in this watershed and this locality and its capture are noteworthy.

***Etheostoma fragi* Distler – Strawberry River Darter.** Little information is available on the biology of this state endemic darter. On 24 April 2016, 4 males in full breeding coloration and running milt were collected from the upper Strawberry River at AR St. Hwy 295 near Byron, Fulton Co. (36.32119°N, 91.938493°W). This observation establishes the Strawberry River Darter as another spring spawner. This fish is listed as a Species of Greatest Conservation Need in Arkansas (Anonymous 2016).

***Etheostoma radiosum* (Hubbs and Black) – Orangebelly Darter.** Scalet (1972, 1973*a*,*b*) provided much information on the life history of *E. radiosum* in Oklahoma; however, no information is available for this species in Arkansas. Spawning typically occurs from late February to mid-April in Oklahoma. We found males in breeding color in the Rolling Fork River off Johnson Bridge Road in Sevier Co. just W of DeQueen (34.064539°N, 94.380613°W) and females with eggs on 1 May 2015, 4 March 2016 and 28 May 2016. Additional reproductive data are: 3 females (35-43 mm TL) from Walnut Creek off Hickorynut Mountain Road, Garland Co. (34.533903°N, 93.371055°W) with eggs on 1 May 2015; one 50 mm TL female from Bear Creek at Bear, Garland Co. (34.534915°N, 93.286449°W) with eggs on the same date; and one 50 mm TL female from Abernathy Spring, Polk Co. (34.468108°N, 93.947656°W) with eggs on 28 May 2016.

***Etheostoma* cf. *spectabile* – Ozark Darter.** Ceas and Page (1997) separated the *E. spectabile* complex of "orangethroat darters" into several species. This undescribed member of the complex is currently being studied by P. A. Ceas (St. Olaf College, Northfield, MN). Little is known about its biology or natural history. On 23 April 2016 at Spring Mill (Big Spring),

S of Cushman off AR St. Hwy 69, Independence Co. (35.828214°N, 91.724288°W), 3 individuals (58-74 mm TL) were collected which were eating scuds (*H. azteca*) and a 64 mm TL female was taken at the same location and date which contained mature eggs.

***Etheostoma squamosum* Distler – Plateau Darter.** In Arkansas, the breeding season of *E. squamosum* extends from March to May (Hubbs and Armstrong 1962). We extend this season to mid-May in the state as 7 females (55-70 mm TL) with eggs were collected on 15 May 2015 from Flint Creek off AR St. Hwy 59 at Gentry, Benton Co. (36.242716N, 94.487408W).

***Percina nasuta* (Bailey) – Longnose Darter.** Little is known of the life history of this uncommon darter in Arkansas. A single male *P. nasuta* was taken from the Middle Fork of the Little Red River just W of Shirley, Van Buren Co. (35.651965°N, 92.320282°W) on 24 April 2016. Robison and Buchanan (1988) observed spawning in the upper White River in mid-May. This male specimen was running milt and thus indicates spawning was in progress, thereby extending the spawning season in Arkansas. This fish is listed as a Species of Greatest Conservation Need in Arkansas (Anonymous 2016).

***Channidae* - Snakeheads**

***Channa argus* (Cantor) – Snakehead.** Any collection of this undesirable, introduced Asian species is noteworthy, thus, we document 2 specimens (330, 440 mm TL) collected on 15 October 2015 off AR St. Hwy 238, SE of Brinkley at Big Piney Creek/Lake Greenlee, Monroe Co. (34.875159°N, 91.166584°W). Neither specimen was found to harbor helminth parasites.

CLASS AMPHIBIA

***Proteidae* – Mudpuppy**

***Necturus louisianensis* Viosca – Red River Mudpuppy.** During 1971, one of us (DGC) collected an adult *N. louisianensis* from Lake Fort Smith, Crawford Co. (35.664627°N, 94.153304°W) whose gills were infested with the monogenean, *Sphyranura oligorchis* Alvey (Fig. 4) (HWML 139186). Alvey (1933) originally described *S. oligorchis* from the common mudpuppy, *N. maculosus* from Pennsylvania and it has also been reported from common mudpuppies from a fish hatchery in Wisconsin (Anonymous 2011). We document the first report of *S. oligorchis* in Arkansas, as well as the first time, to our knowledge, from the Red River Mudpuppy.

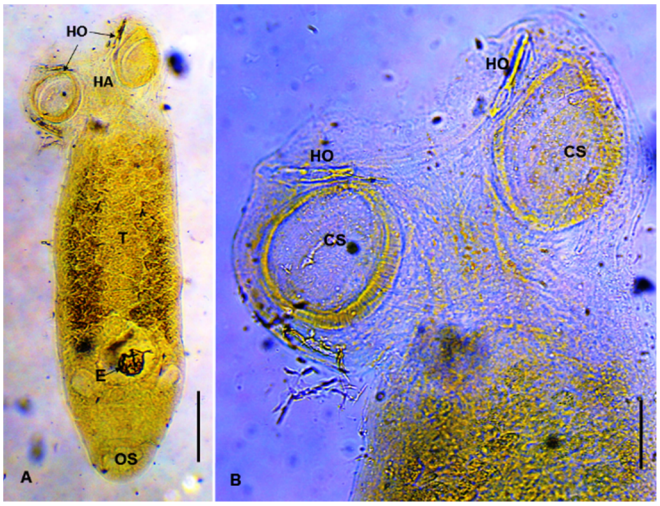

Figure 4. *Sphyranura oligorchis* from *Necturus louisianensis*. A. Whole specimen showing egg (E), haptor (HA), hooks (HO), and oral sucker (OS). Scale bar = 175 µm. B. Closeup of same showing caudal suckers (CS) and hooks (HO). Scale bar = 50 µm.

***Hylidae* – Tree Frogs**

***Hyla squirella* Bosc in Daudin – Squirrel Treefrog.** This small hylid frog, found throughout the southeastern United States, was only recently discovered in Arkansas, in Union Co. (Fulmer and Connior 2013; Connior et al. 2014). The closest known record was circa 80 km away in nearby Ouachita Parish, Louisiana (Dundee and Rossman 1989). We report a photovouchered new record of the squirrel treefrog, collected by E. Burke on 10 March 2017 from adjacent Ashley Co. at Overflow NWR (33.148202°N, 91.597677°W).

CLASS MAMMALIA

ORDER SORICOMORPHA

***Soricidae* - Shrews**

***Blarina carolinensis* (Bachman) – Southern Short-tailed Shrew**. On 14 May 2016, Bill and Vanessa Bateman found a dead piebald specimen of *B. carolinensis* near their home in Alpine, Clark Co., AR (Fig. 5). Among shrews, only albino least shrews (*Cryptotis parva*) have been reported in Arkansas previously (Sealander 1981).

ORDER LAGOMORPHA

***Leporidae* – Hares and Rabbits**

***Sylvilagus floridanus* (JA Allen) – Cottontail Rabbit.** Larvae of bot flies (*Cuterebra* sp.) cause myiasis in the animals they infest, and near maturity appear as large, darkened maggots visible through a hole

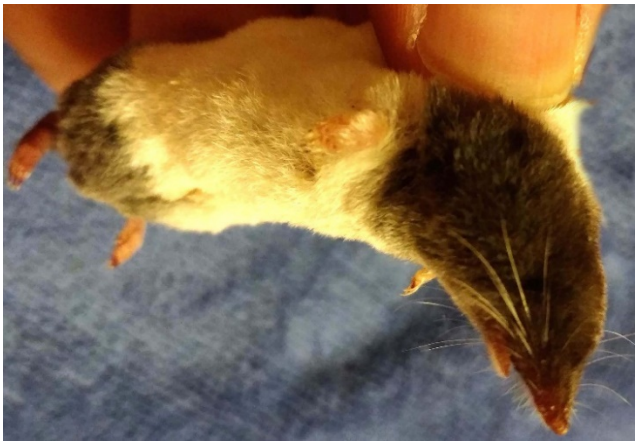

Figure 5. Piebald specimen of *Blarina carolinensis* from Clark Co., 14 May 2016. Photo by B. and V. Bateman.

in the skin of the host. Though bot flies in rabbits may be common (reported in 24% of cottontails in Virginia [Jacobson et al. 1978] and up to 50% in Wisconsin [Haas and Dicke 1958]), they have not been reported in cottontails in Arkansas. We found a bot fly in the neck of a cottontail collected 8 October 2016 in Hot Spring Co., on Rainbow Road W of Bismarck. We expect such parasitism to be common, but no reports are available of frequency or occurrence in Arkansas.

ORDER CHIROPTERA

***Vespertilionidae* – Vesper Bats**

***Corynorhinus rafinesquii* (Lesson) – Rafinesque's Big-eared Bat**. Little is known of the reproductive biology of this bat in Arkansas (Sealander and Heidt 1990). On 5 June 2016, investigation of a dilapidated abandoned house located in Ouachita Co. near the intersection of AR St. Hwy 57 and Ouachita Co. Rd. 517 (33.566888°N, 93.094835°W) revealed a nursery colony of at least 5 female *C. rafinesquii*, each attending a single volant offspring. At the time, a black rat snake (*Pantherophis obsoletus*) was found over a door, attempting to prey on the colony.

***Myotis sodalis* Miller and GM Allen – Indiana Bat**. We report previously undocumented museum specimens of this endangered species: a male and female collected during February 1935 in Izard Co. at Calico Rock (Univ. Michigan Museum of Zoology 75494, 75495). A new county record was observed on 2 November 2016, when a juvenile female was captured after flying into glass doors in Jonesboro, Craighead Co. (35.83801°N, 90.70072°W). The bat was banded and released.

***Myotis grisescens* AH Howell – Gray Bat**. A new county record is represented by two adult male gray bats captured on 13 April 2016 in a bridge over the Mulberry River, Johnson Co.

***Etesicus fuscus* (Palisot de Beauvois) – Big Brown Bat**. A post-lactating female was captured in a mist net set in a bottomland hardwood forest on 10 July 2015. This new county record for Prairie Co. was taken at the southern end of the Cache River NWR (34.79655°N 91.37780°W).

A new county record representing Hempstead Co. was obtained on 16 July 2016 when 6 female, 1 male, and 1 unsexed individual were captured in a mist net placed over a firelane on the Hope Upland Wildlife Management Area, Sec. 31, T11S, R24W.

***Cricetidae* – New World Mice**

***Peromyscus attwateri* Allen – Texas Deermouse**. A specimen captured on 15 May 2016 from 13 km NE Berryville, Carroll Co., partially fills a distributional hiatus in northwestern Arkansas (Sealander and Heidt 1990). The habitat was a rocky cedar glade.

ORDER CARNIVORA

***Felidae* – Cats**

***Lynx rufus* (Schreber) – Bobcat.** An adult male *L. rufus* was collected in Hot Spring Co. on 25 November 2013 on AR St. Hwy 84, 4.8 km E jct. AR St. Hwys 7 and 84 in Bismarck. It was found to be infested with 11 blacklegged ticks (*Ixodes scapularis* Say; 4 males, 7 females). This is the second time *I. scapularis* has been reported from a bobcat in Arkansas but the first time a specific locality has been provided (see McAllister et al. 2016*b*).

***Canidae* – Canids**

***Canis latrans* Say – Coyote.** A juvenile male *C. latrans* was collected on 11 October 2016 from 8.5 km S of Arkadelphia, Clark Co. (34.05167°N, 93.09928°W). This coyote was infested with 12 Gulf Coast ticks (*Amblyomma maculatum* (Koch); 10 males, 2 females). Adults feed on a variety of large mammals such as deer and cattle whereas immatures feed on smaller mammals and on birds (Cooley and Kohls 1944, Teel et al. 2010). Although there are previous records of this tick on domestic dogs (*C. familiaris*) in the state (McAllister et al. 2016*b*), this is the first time this tick has been found on *C. latrans* from Arkansas.

***Mustelidae* – Weasels and allies**

***Taxidea taxus* (Schreber) – American Badger.** In recent years, badgers have established populations in northeastern Arkansas and reproduction has been reported in Crittenden Co. (Tumlison and Sasse 2015).

We have a recent report of a male badger injured by a collision with a car on 16 December 2016 on AR St. Hwy 50 and Woollard Road in Crittenden Co. (35.25642°N, 90.32569°W). The animal was caught and photographed by a person who commented that he often sees badgers along the highway. Though this observation is only 7.3 km (4.5 mi.) NW of the nearest reported location in the county, it further documents the presence and distribution of this rare mustelid, which is listed as a Species of Greatest Conservation Need in Arkansas (Anonymous 2016).

Other than new records of distribution, little is known about biology of badgers in Arkansas. Examination of an adult female *T. taxus* collected on 11 June 2014 from 5.5 km WNW of Marion, Crittenden Co. (35.22627°N, 90.25420°W) revealed several nematodes, *Physaloptera torquata* Leidy (HWML 99823) in its stomach and colon. Although *P. torquata* has been reported previously from badgers from Iowa, Kansas, Minnesota, South Dakota, and Texas (see Pence and Dowler 1979), this is the first time the parasite has been reported from a badger in Arkansas.

ORDER ARTIODACTYLA

***Cervide* – Deer**

***Odocoileus virginianus* (Zimmerman) – White-tailed Deer.** White-tailed deer very rarely possess upper canine teeth, though they are present in elk. On 16 October 2016, N. R. Cain harvested a buck N of Crystal Springs, Garland Co., estimated by tooth wear to be 3-4 years old, with bilateral presentation of upper canine teeth (Fig. 6). This condition is considered to be atavistic.

Figure 6. Skull of *O. virginianus* from Garland Co. with atavistic appearance of upper canines. Inset shows detail of the tooth.

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An Annotated Checklist of the Crayfishes (Decapoda: Cambaridae) of Arkansas

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Running Title: Crayfishes of Arkansas

Abstract

Prior to the present study, 56 species with 3 additional subspecies for a total of 59 different taxa of crayfishes were recognized from Arkansas. We add a single species (Carmel Crayfish, *Fallicambarus schusteri*) to that list, subtract a documented synonym (*Procambarus ferrugenius = Procambarus liberorum)*, update the classification to better reflect recent phylogenetic insights, and provide an updated annotated checklist of the 59 crayfish taxa of presently known from the state. There are 8 endemic species in Arkansas, including the Bayou Bodcau Crayfish (*Bouchardina robisoni*), Boston Mountains Crayfish (*Cambarus causeyi*), Hell Creek Cave Crayfish (*C. zophonastes*), Jefferson County Crayfish (*Creaserinus gilpini*), Ouachita Burrowing Crayfish (*Fallicambarus harpi*), Slenderwrist Burrowing Crayfish (*F. petilicarpus*), Saline Burrowing Crayfish (*F. strawni*), and Redspotted Stream Crayfish (*Faxonius acares*). There are also 2 federally endangered species, the Benton County Cave Crayfish (*Cambarus aculabrum*) and the Hell Creek Cave Crayfish (*C. zophonastes*) that inhabit Arkansas karst habitat. We expect that additional species will be included in the list with further DNA analyses.

Introduction

Crayfishes are a taxonomically diverse group of decapod crustaceans with over 669 species worldwide and 2 centers of diversity, one in the southeastern Appalachian Mountains of the southeastern United States (Northern Hemisphere center) and one in south-east Australia (Southern Hemisphere center) (Crandall and Buhay 2008; Crandall 2016; Crandall and De Grave 2017). Crayfishes are a monophyletic group of arthropods that is a sister group to the clawed lobsters (Nephropoidae Dana, 1852) (Crandall et al. 2000; Bracken-Grissom et al. 2014).

In Arkansas, crayfishes can serve as keystone species and are an integral component of the state's aquatic ecosystems. Fishes, particularly sunfishes and basses (family Centrarchidae) may consume up to two-thirds of the annual production of crayfishes in many streams (Taylor et al. 1996). Crayfishes contribute to the maintenance of food webs by processing vegetation and leaf litter (Huryn and Wallace 1987; Griffith et al. 1994), which increases the availability of nutrients and organic matter to other aquatic and terrestrial organisms.

Crayfishes are members of the Phylum Arthropoda, or joint-legged animals, which includes 97 to 99% of all the animals on Earth. They are classified as crustaceans because of the 2 pair of antennae they possess and the fact they breathe by gills. Individuals are protected by a heavily armored exoskeleton and have 5 pairs of walking legs, the first of which function as enlarged pincers (chelipeds).

Prior to this study, Arkansas had been known to support 59 crayfish taxa (Bouchard and Robison 1980; Taylor et al. 1996), all belonging to the family Cambaridae, and grouped into 7 genera. Our current study also recognizes 59 taxa representing 8 genera based on: (1) 45+ years of fieldwork in Arkansas by one of us (HWR) from 1971 to 2017, (2) a careful search of the pertinent literature, and (3) a search of museums that house Arkansas crayfish specimens. The purpose of this study is: (1) to provide a checklist of all crayfish species/subspecies presently known to occur in Arkansas with an updated phylogenetically-based taxonomy, (2) include a brief account of the habitat of each state crayfish, and (3) establish the state distributions for all known Arkansas crayfishes. We desire to provide this annotated checklist so that aquatic biologists, naturalists, interested laymen, government scientists, and resource managers involved in environmental work in the state would have a useful document to consult in the interim while HWR and KAC prepare a field guide to the crayfishes of Arkansas,

currently in progress.

Materials and Methods

Fieldwork was carried out during a 45+ yr period from 1971 to July 2017 in all seasons, but particularly in the spring, summer, and fall when collecting is best for crayfishes. Over 1,000 personal collections of crayfishes in Arkansas have been made by HWR, plus numerous collections in the state made by CTM, KAC, and the late HH Hobbs, Jr. (1914–1994), the latter who first guided HWR into the study of Arkansas crayfishes. In addition, collections of Arkansas crayfish housed at Southern Arkansas University (SAU), the Smithsonian National Museum of Natural History (USNM 2016), and the Illinois Natural History Survey (INHS 2016) were also examined.

The 59 taxa listed herein are known to inhabit Arkansas and grouped together in the family Cambaridae using the updated classification scheme of Crandall and De Grave (2017) which better reflects evolutionary associations of crayfish species. The Appendix serves as a convenient checklist of the crayfishes of Arkansas for biologists, naturalists, and resource managers.

Distribution is usually expressed in terms of sections of the state (e.g., northern, southwestern, and central). In some instances, distribution is stated in terms of specific drainage basins such as the Ouachita River system (Fig. 1). If the species is known from only one or 2 streams or counties, the names of the stream and county are given. Statements regarding distributional range within the state are, for the most part, based on collections made by HWR during his longtime statewide collecting effort. Williams (1954), Reimer (1963), Bouchard and Robison (1980), Hobbs and Robison (1985, 1989), and additional published literature records for Arkansas were also examined. Those records and others are housed in the Arkansas Crayfish Database (ACD) held by the Arkansas Game and Fish Commission. Conservation status of Arkansas crayfishes is taken from Taylor et al. (2007) of which HWR was a member of the original AFS Committee and supplied data for the determination of Arkansas crayfishes used in the publication, as well as IUCN (2016) Red List status where KAC participated in Red List assessments (Richman et al. 2015). In addition to those species documented to occur within the political boundaries of Arkansas, we also provide a list of problematic species that have been formerly listed from Arkansas and/or may occur within state borders.

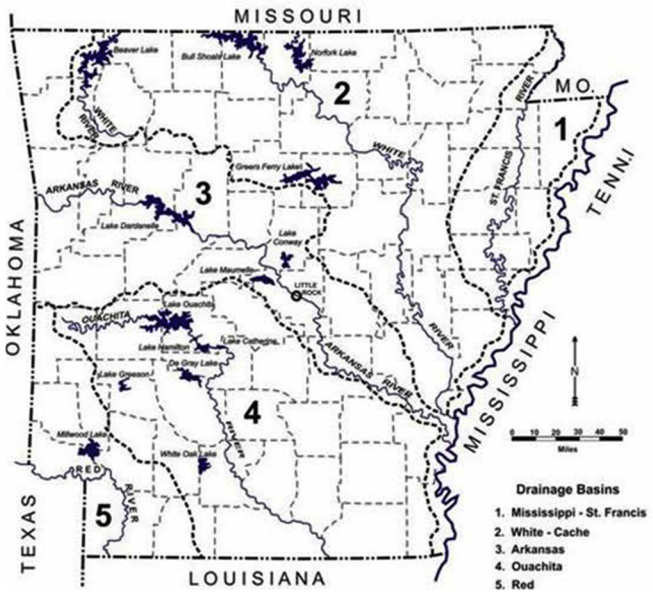

Figure 1. Five major drainage basins of Arkansas. From: http://www.geology.ar.gov/water/surface_water.htm.

Results and Discussion

Fifty-nine crayfish taxa are known to occur in Arkansas. The following is an annotated list of those species, as well as information on their geographic distribution in the state, ecology, and natural history.

Basic Life History Cycle

Although highly variable, most crayfishes in Arkansas mate between September and March. Form I males (reproductively active with well-defined terminal elements of the first pleopods) seek out receptive females and mating is accomplished. Sperm are carried by the female until oviposition (egg-laying) which may be in March, April and May, although some species begin as early as December or January (Page 1985). Following oviposition, the eggs are attached to the abdomen of the females and they are said to be ovigerous or "in berry." Females carry the eggs for 2 to 20 weeks depending on the water temperature (Page 1985). After hatching, young move quickly through a series of molts until sexual maturity is reached by late summer or early fall.

Taxonomic Considerations

The taxonomy of North American crayfishes is based on numerous morphological characteristics (Hobbs 1972a), the secondary sexual characters being

of primary importance, such as the annulus ventralis, copulatory hooks, bosses on the coxae of some periopods (=walking legs), and first pleopods (Bouchard and Robison 1980). The single most important character in identifying most species of and practically all of the genera of North American crayfishes is the morphology of the male first pleopods. In addition, another important feature for identification, particularly those in the genus *Cambarus*, is the chelae. The taxonomy of freshwater crayfishes was recently updated based on the last 20 years of phylogenetic studies that have called into question family, subfamily, genus, and subgenus affiliations for various taxa (Crandall and De Grave 2017). We have followed their updated classification, which reflects the evolutionary history of the crayfishes.

In crayfishes of the family Cambaridae, adult males exhibit 2 morphological forms during the year, molting into these conditions with only the first form (form I) males capable of breeding, the second form, or form II male being sexually nonfunctional (Bouchard and Robison 1980). The first pleopod, or gonopod as it is known, of the form I male with its delicate, finely sculptured elements, at least one of which consists of amber, corneous material, is easily distinguished from the form II gonopod which has elements usually reduced in length and/or more inflated and without a corneous deposit (Hobbs 1972a). Also reduced in size are the remaining secondary sexual characters such as the chelae.

Ecological Categories

Arkansas crayfishes occupy 4 main habitat types: (1) primary burrowers (those crayfish who spend their entire life cycles in burrows), (2) stream-dwellers, (3) pond/lake/large river dwellers including secondary burrowers (who do require connectivity of burrows with freshwater), and (4) stygobitic species (obligate cave-dwellers) (Crandall and Buhay 2008).

Brief Historical Review

The earliest publication dealing with Arkansas crayfishes was Hermann Hagen's (1870) monograph on North American crayfishes which listed *Cambarus obesus* Hagen (= *C. diogenes*) from Arkansas. For the next 70 to 80 years, from 1870 until the late 1940s and 1950s, few additional references to Arkansas crayfishes appeared in the scientific literature. The cornerstone of any serious study of Arkansas crayfishes is A.B. Williams' (1954) study of crayfishes of the Ozark and Ouachita Mountain uplands of Arkansas and Missouri. He discussed in detail the various forms he collected in these regions and provided numerous new collecting sites. Unfortunately, it is now badly outdated and the taxonomy has changed. Rollin Reimer's M.S. thesis (1963) at the University of Arkansas provided an unpublished survey of the crayfishes of Arkansas which greatly assisted the identification and study of those in the state. He made 289 collections containing 7,300 specimens representing 33 species in 4 genera and also included the first state checklist and brought the number of species known in 1963 from Arkansas to 37. Fitzpatrick (1978) described the primary burrower, *Procambarus liberorum*, from near Fayetteville, Arkansas, a species that Reimer (1969) almost certainly had listed in his doctoral dissertation. Later, Bouchard and Robison (1980) summarized the available information on the crayfishes of Arkansas and provided the first published inventory of the state crayfishes listing 51 taxa (47 species and 4 subspecies). In his doctoral work on crayfishes, Crandall (1993) studied the molecular systematics and evolutionary biology of the crayfish subgenus *Procericambarus* which included numerous Ozarkian species from Arkansas resulting in the first DNA sequence based phylogeny of freshwater crayfish (Crandall and Fitzpatrick 1996). In adjacent Missouri, Pflieger's (1996) book on the *Crayfishes of Missouri* provided fine pen and ink line drawings and color photographs of the 35 crayfish species living there, a number of which also occurred in Arkansas, thus identification of Arkansas taxa was made easier using his photos and line drawings.

In a series of papers on Arkansas crayfishes, Hobbs and Robison (1982, 1985, 1988, 1989) described several new species of *Procambarus* and *Fallicambarus* from Arkansas, as well as summarized data on the subgenus *Girardiella* of *Procambarus* and *Fallicambarus* of the state. More recently, study of the Arkansas crayfish fauna has accelerated during the past 2 decades as studies by Robison and Leeds (1996), Robison (1997, 2001), Dukat and Magoulick (1999), Flinders and Magoulick (2003, 2007), Robison and Crump (2004), Robison and Wagner (2005), Graening et al. (2006a, 2006b, 2006c, 2006d), Robison and McAllister (2006, 2008, 2010, 2014), Rabalais and Magoulick (2006), Westhoff et al. (2006), Magoulick and DiStefano (2007), Larson and Magoulick, (2008, 2011), Robison et al. (2009, 2014, 2017), McAllister and Robison (2010, 2012), Tumlison and Robison (2010), Wagner et al. (2010a, 2010b), McAllister et al. (2011), Ainscough et al. (2013), Taylor and Robison (2016), and Tumlison et al. (2017) all have examined aspects of the Arkansas

crayfish fauna, provided distributional data on state species, and/or described new species occurring in the state.

Conservation of North American crayfishes was aided by the original publication of Taylor et al. (1996) and followed a decade later by Taylor et al. (2007) which provided the current status for all North American crayfishes including those inhabiting Arkansas. Additionally, Richman et al. (2015) provided a global assessment of conservation status of the freshwater crayfishes using international criteria and include assessments of all the Arkansas crayfish.

Annotated Checklist of Arkansas Crayfishes

Worldwide, there are currently over 669 described species of freshwater crayfishes with an average of 5 to 10 species still being described each year (Crandall and Buhay 2008; Crandall and De Grave 2017). Over 404 (60%) of these are found in the United States and Canada (Taylor et al, 1996). In Arkansas, we have documented 56 species of crayfishes with 3 subspecies represented, thus a total of 59 crayfish taxa inhabiting the state. Of the 59 crayfish taxa listed herein, all but 3 (*Cambarus aculabrum*, *Cambarus setosus*, and *Faxonius cyanodigitus*) have been personally collected in Arkansas by HWR (KAC has collected the *Cambarus* species). All crayfish occurring in Arkansas currently belong to the family Cambaridae.

Within Arkansas, the genus *Faxonius* slightly dominates the crayfish fauna with 18 species and 3 subspecies, while the genus *Procambarus* is represented by 16 species, followed by *Fallicambarus* and *Cambarus* with 7 species each, *Creaserinus* with 3 species, *Cambarellus* and *Faxonella* with 2 species each, and the monotypic genus *Bouchardina* with a single species. In addition, we have discovered several undescribed species of crayfishes in the state (e.g., Crandall et al. 2009); however, formal descriptions of these new species have not yet been completed.

PHYLUM ARTHROPODA VON SIEBOLD 1848
SUBPHYLUM CRUSTACEA BRÜNNICH 1772
CLASS MALACOSTRACA LATREILLE 1802
ORDER DECAPODA LATREILLE 1802
FAMILY CAMBARIDAE HOBBS 1942
GENUS *BOUCHARDINA* HOBBS 1977

***Bouchardina robisoni* Hobbs 1977 - Bayou Bodcau Crayfish**

Bouchardina robisoni (Fig. 2) inhabits lentic and sluggish lotic habitats, especially the backwaters of Bayou Bodcaw (=Bodcau) (Red River drainage) and lower Bayou Dorcheat in Columbia, Hempstead, Howard, Lafayette, and Nevada counties of southwest Arkansas (Robison and McAllister 2010). This species has been collected in shallow and small intermittent streams with a sandy substrate and aquatic vegetation such as water primrose (*Ludwigia*), bladderwort (*Utricularia*), and submerged grasses (Robison and McAllister 2010). It is an Arkansas endemic (Robison and Allen 1995). IUCN Red List Status: Data Deficient.

Figure 2. Bayou Bodcau Crayfish, *Bouchardina robisoni*.

GENUS *CAMBARELLUS* ORTMANN 1905

***Cambarellus (Pandicambarus) puer* Hobbs 1945 - Swamp Dwarf Crayfish**

This tiny crayfish is found in well vegetated swamps, ditches, lakes, ponds, sloughs, and sluggish streams with muddy substrate (Hobbs 1989). Although it is rarely collected in the Coastal Plain of Arkansas, elsewhere in its range, this crayfish is a widespread, generalist species, which is believed to be abundant and has no known threats. Tumlison et al. (2017) recently documented the first report of an ovigerous female in Arkansas as well as new collections from Calhoun, Cleveland, Columbia, Greene, Howard, Jackson, Lafayette, Monroe, Union, and White counties. IUCN Red List Status: Least Concern.

***Cambarellus (Pandicambarus) shufeldtii* (Faxon 1884) - Cajun Dwarf Crayfish**

This dwarf crayfish (Fig. 3) occupies ditches, sloughs, oxbow lakes, swamps, and sluggish streams (Hobbs 1989). It has been known to burrow when water levels are low. In Arkansas, it has been taken only from the Coastal Plain. The first report of ovigerous females in the state was documented by Tumlison et al. (2017) as well as new collections from Columbia, Jackson, Lafayette, Lawrence, White, and Woodruff counties. IUCN Red List Status: Least Concern.

Figure 3. Cajun Dwarf Crayfish, *Cambarellus shufeldtii*.

GENUS *CAMBARUS* ERICHSON 1846

Cambarus aculabrum Hobbs and Brown 1987 - Benton County Cave Crayfish

Known only from 4 caves (Bear Hollow, Elm Springs, Logan, and Old Pendergrass) in and around Benton and Washington counties, this federally endangered cave troglobitic crayfish lives in subterranean streams (Hobbs and Brown 1987; Graening et al. 2006d). The type locality, Logan Cave (Benton County), part of the federally-protected Logan Cave National Wildlife Refuge, is a dendritic stream channel cave located in the Mississippian cherty-limestone, Boone Formation of the Springfield Plateau (Hobbs and Brown 1987). Since 2004, extensive survey efforts nearby have revealed no other specimens. The primary reason for federal listing of the species and still remains a serious threat is habitat degradation from groundwater pollution (Graening et al. 2006d). It is listed as critically imperiled (S1) in Arkansas according to NatureServe (2015). IUCN Red List Status: Critically Endangered.

Cambarus causeyi Reimer 1966 - Boston Mountains Crayfish

This primary burrowing crayfish inhabits complex burrows near spring and run-off areas in upland environs (Robison and Leeds 1996). It is an Arkansas endemic known from the Arkansas River drainage in Franklin, Johnson, Madison, Newton, Pope, Searcy, and Stone counties (Robison and Allen 1995; Robison and Leeds 1996). This species is also known from springs in the Boston Mountains and from 8 watersheds (Upper White, Buffalo, Mulberry, and Upper Mulberry rivers, Spadra, Little Piney, and Big Piney creeks, and the Middle Fork of Illinois Bayou). It may also be present in 10 more watersheds in the Ozark National Forest (Robison and Leeds 1996). IUCN Red List Status: Least Concern.

Cambarus diogenes Girard 1852 - Devil Crawfish

Originally considered a subspecies, this primary burrower (Fig. 4) lives in large burrows with tall mud chimneys near ponds, streams, or ditches on the more northerly portion of the Coastal Plain. It can be excavated almost anywhere where the water table is near the surface (Pflieger 1996). This is a broadly distributed species (across the eastern US) and may be a species complex and, therefore, is currently under investigation to define species limits through its range. Tumlison et al. (2017) reported the first specimens from Lawrence County. IUCN Red List Status: Least Concern.

Figure 4. Devil crawfish, *Cambarus diogenes*.

Cambarus hubbsi Creaser 1931 – Hubbs' Crayfish

An uncommon stream crayfish in Arkansas, *C. hubbsi* has been found in riffles and runs of streams, burrows, and caves (Hobbs 1989). It occurs in northeastern Arkansas in the Eleven Point, Spring, Strawberry, and St. Francis river drainages and portions of the White River drainage (Flinders and Magoulick 2007). IUCN Red List Status: Least Concern.

Cambarus ludovicianus Faxon 1884 - Painted Devil Crayfish

This is a rather striking dark blue primary burrower that inhabits large burrows in lotic habitats on the Coastal Plain of southern and southwestern Arkansas. Young *C. ludovicianus* have been found in Nix Creek, Texarkana, Miller County (McAllister *unpubl*.). The

painted devil crayfish has been reported as one of the most secretive crayfishes in the Mississippi River drainage as it only leaves its burrows at night or during rainy conditions (Reimer and Clark 1974). Tumlison et al. (2017) documented the first report of an ovigerous *C. ludovicianus* from the state as well as new collections from Columbia and Lafayette counties. IUCN Red List Status: Least Concern.

***Cambarus setosus* Faxon and Garman in Garman, 1889 - Bristly Cave Crayfish**

Graening et al. (2006a) added *C. setosus* to the state list rather recently. This troglobitic species is known in Arkansas from only 2 widely separated caves in Benton and Independence counties, respectively, in northern Arkansas. However, it is also known from at least 40 sites in Missouri (Pflieger 1996), many with declining populations, all restricted to cave environments and most are not adequately protected. The population in Benton County may be at risk because the habitat is located in a watershed that contains several municipal sewage treatment outfalls and numerous confined animal feeding operations (Graening et al., 2006a). It is listed as S1 (critically imperiled) in Arkansas (NatureServe 2015). IUCN Red List Status: Near Threatened.

***Cambarus zophonastes* Hobbs and Bedinger 1964 - Hell Creek Cave Crayfish**

Hobbs and Bedinger (1964) originally described this cave crayfish from Hell Creek Cave, Stone County. An Arkansas endemic (Robison and Allen 1995), it was later discovered in a second Stone County locality, Nesbitt Spring Cave (Graening et al. 2006b, 2006c). In its cave environment, *C. zophonastes* has been observed on the sides of steep rock sides and on the mud bottom of the cave stream (Hobbs and Bedinger 1964, HWR *pers. observ*.). This species was designated as federally endangered in the U.S. in 1987 and listed as S1 (critically imperiled) in Arkansas by NatureServe (2015). It is threatened by a variety of negative factors including: groundwater pollution, a variety of human disturbance, and a reduction in nutrient availability. IUCN Red List Status: Critically Endangered.

GENUS *CREASERINUS* HOBBS 1973

***Creaserinus caesius* (Hobbs 1975) - Timberlands Burrowing Crayfish**

Creaserinus caesius is a widespread primary burrower found in the basins of the Ouachita River and Bayou Dorcheat in southern Arkansas (Robison and Allen 1995). It inhabits roadside ditches with a high water table, and clay-gravel substrates. IUCN Red List Status: Least Concern.

***Creaserinus fodiens* (Cottle 1863) - Digger Crayfish**

This crayfish is a wide-ranging variable form (Fig. 5) that occupies lentic and lotic habitats as well as semi-terrestrial burrows in fine clay soils on the Coastal Plain. It can be found in a range of habitats such as wetlands (marshes and swamps), roadside ditches, creek banks and among rooted semi-aquatic plants and grasses (Hamr 2005; Taylor et al. 2005) but does not tolerate fast-flowing streams (Bouchard 1974). Tumlison and Robison (2010) reported new county records for *C. fodiens* in Chicot, Clark, and Ouachita counties. Due to the variability and broad distribution of these species, it is thought to be a species complex and is currently under investigation. IUCN Red List Status: Least Concern.

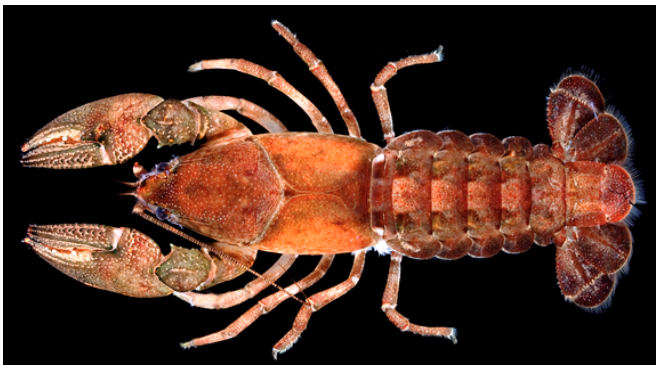

Figure 5. Digger Crayfish, *Creaserinus fodiens*.

***Creaserinus gilpini* (Hobbs and Robison 1989) - Jefferson County Crayfish**

This crayfish is another primary burrower and has been taken only from complex burrows consisting of branching galleries, several of which, except in dry seasons, reach the surface, some of their openings marked by rather crudely constructed turrets (Hobbs and Robison 1989). Thus far, this state endemic has been collected only in Cleveland and Jefferson counties of southcentral Arkansas (Robison and Wagner 2005). IUCN Red List Status: Near Threatened.

GENUS *FALLICAMBARUS* HOBBS 1969

***Fallicambarus dissitus* (Penn 1955) - Pine Hills Digger**

In Arkansas, this primary burrower is known only from burrows in the Red and Ouachita River watersheds in Columbia and Union counties. This species is found in complex burrows, approximately 61 cm (2 ft) deep, in roadside ditches consisting of sandy clay substrate

(Hobbs and Robison 1989). IUCN Red List Status: Data Deficient.

***Fallicambarus harpi* Hobbs and Robison 1985 - Ouachita Burrowing Crayfish**

Fallicambarus harpi is a primary burrower in ditches, lawns, fields, and pastures. Robison and Crump (2004) investigated the distribution, natural history aspects, and its status and found the height of burrowing activity to occur in April when individuals dig burrows ranging from 45 to 85 cm deep and chimneys up to 20 cm in height. Soils tend to consist of sandy clay with organic material with grasses and sedges abundant (Hobbs and Robison 1985). Hundreds of these burrows can occupy a single pasture at a given time. Currently, this crayfish is known only from the Ouachita River basin in Garland, Hot Spring, Montgomery, and Pike counties and, as such, is an Arkansas endemic (Robison and Crump 2004; Robison et al. 2008). IUCN Red List Status: Near Threatened.

***Fallicambarus jeanae* Hobbs 1973 - Daisy Burrowing Crayfish**

This bluish-colored primary burrower is found throughout the Ouachita River basin in Clark, Hot Spring, Montgomery, and Pike counties. It is specifically found in roadside ditches and low-lying seepage areas with sandy to clay soils. IUCN Red List Status: Vulnerable.

***Fallicambarus petilicarpus* Hobbs and Robison 1989 - Slenderwrist Burrowing Crayfish**

Originally described by Hobbs and Robison (1989) from a single locality in western Union County, Arkansas, this primary burrower and Arkansas endemic has subsequently been collected in adjacent Columbia County in the extreme southern part of the state. This species is presently known from only 18 specimens, from 2 collections at the type locality, and an unknown number of specimens at a second locality in Columbia County (Robison 2001; Tumlison and Robison 2010). Tumlison and Robison (2010) reported that specimens were dug from complex burrows ranging from 20 to 48 cm (8 to 19 in.) in roadside ditches or seepage areas with rushes (*Juncus* sp.) common. IUCN Red List Status: Endangered.

***Fallicambarus schusteri* Taylor and Robison 2016 – Carmel Crayfish**

The Carmel Crayfish (Fig. 6) is the most recently described crayfish in Arkansas. Taylor and Robison (2016) described *F. schusteri* from the flatlands draining south into the Red River from Idabel in southeastern McCurtain County, Oklahoma, to Ashdown in southcentral Little River County, Arkansas. The species occurs in roadside ditches that seasonally flood and have silt and silt-loam dominated soils. A single collection of this primary burrower is known from Arkansas at a roadside ditch *ca.* 0.8 km SW of Ashdown (33.86523°N, 94.1368°W) taken on 23 April 2015 and deposited in the INHS. IUCN Red List Status: Data Deficient.

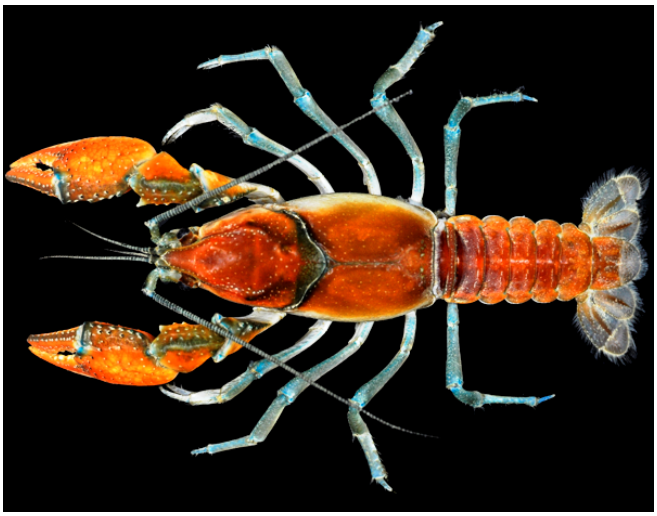

Figure 6. Carmel Crayfish, *Fallicambarus schusteri*.

***Fallicambarus strawni* (Reimer 1966) - Saline Burrowing Crayfish**

This crayfish is an Arkansas endemic and primary burrower and has been found in the marshy areas drained by the Saline River (Red River drainage) in Howard, Pike, and Sevier counties. Its preferred substrate is sandy-clay; nearby streams are clear, fast-running, and shallow with rocky substrate. IUCN Red List Status: Least Concern.

***Fallicambarus tenuis* (Hobbs 1950) - Ouachita Mountain Crayfish**

Fallicambarus tenuis inhabits burrows and freshwater springs, or can be found under rocks in small first and second order clear cool permanent streams in the Ouachita Mountains. Robison et al. (2009) also reported it from the Arkansas and Red River basins in western Arkansas. IUCN Red List Status: Data Deficient.

GENUS *FAXONELLA* CREASER 1933

***Faxonella blairi* Hayes and Reimer 1977 - Blair's Fencing Crayfish**

Prior to the report by Robison et al. (2014), this

small crayfish was thought to be rare in Arkansas. Their study documented 87 collections of over 900 specimens from lentic habitats such as roadside ditches in southwestern Arkansas in the Little and Red River basins of Columbia, Howard, Little River, Miller and Sevier counties. In addition, phylogenetic analyses clearly showed that *F. blairi* and *F. clypeata* form reciprocally monophyletic groups and are genetically differentiated from one another and from species in other genera (Robison et al. 2014). IUCN Red List Status: Least Concern.

***Faxonella clypeata* (Hay 1899) - Ditch Fencing Crayfish**

A Coastal Plain inhabitant in Arkansas, *F. clypeata* (Fig. 7) occurs in sluggish streams, lentic habitats, and occasionally burrows as a tertiary burrower (Hobbs 1989). Tumlison and Robison (2010) collected specimens of *F. clypeata* using aquatic dip nets from lentic bodies of water with substrates of decaying leaves. Robison and McAllister (2014) documented *F. clypeata* from 1,198 specimens collected from 34 of 75 (45%) counties in the state. IUCN Red List Status: Least Concern.

Figure 7. Ditch Fencing Crayfish, *Faxonella clypeata*.

GENUS *FAXONIUS* ORTMANN 1905

***Faxonius acares* (Fitzpatrick 1965) - Redspotted Stream Crayfish**

This stream crayfish inhabits rapidly flowing water associated with shoals and spring outflows also being favored (McAllister and Robison 2010). Its range includes tributaries of the Ouachita River system in Clark, Garland, Hot Spring, Montgomery, Perry, Pike, Polk, and Saline counties (McAllister and Robison 2010). This is a true Arkansas endemic (Robison et al. 2008). IUCN Red List Status: Least Concern.

***Faxonius cyanodigitus* (Johnson 2010) - Red River Painted Crayfish**

Only one collection of this recently described crayfish from Texas and Arkansas has been documented from the state. The single Arkansas record (Johnson 2010) of this species is a Form I male collected on 13 October 2007 from the Red River at St. Hwy. 59, Little River County (33.55113°N 94.04125°W). IUCN Red List Status: Data Deficient.

***Faxonius difficilis* (Faxon 1898) - Painted Crayfish**

This poorly known crayfish is known in Arkansas only from rocky streams in Washington County. This species inhabits a wide variety of stream types from small to moderate streams with clear water and white sand bottoms, to large streams with mud bottoms and very silty water. This taxonomic group is badly in need of study in Arkansas and adjacent Oklahoma. IUCN Red List Status: Least Concern.

***Faxonius eupunctus* (Williams 1952) - Coldwater Crayfish**

This is a rarely encountered crayfish in the Eleven Point and Spring River systems and the Strawberry River drainage, near Evening Shade in northern Arkansas. Hobbs (1989) reported the habitat of *F. eupunctus* as clear, cold, rapid streams with coarse gravel substrates. It is often found in deeper pools from 2.5 to 3 m under large pieces of cobble. It appears to have been displaced from a portion of its range by the recently introduced crayfish, *F. neglectus* (Larson and Magoulick 2008). IUCN Red List Status: Vulnerable.

***Faxonius lancifer* (Hagen 1870) - Shrimp Crayfish**

This crayfish (Fig. 8) inhabits sluggish streams and lentic habitats in 19 counties of the Coastal Plain in Arkansas. Robison et al. (2017) recently provided a summary of biological information on *F. lancifer* in the state. IUCN Red List Status: Least Concern.

***Faxonius leptogonopodus* (Hobbs 1948) - Little River Creek Crayfish**

Another small stream inhabitant, *F. leptogonopodus* lives in small, clear, rocky streams in the Little River system (Red River Drive) in the Ouachita Mountains of southwestern Arkansas. This species is found in fast flowing water and is also a tertiary burrower (Williams 1954). IUCN Red List Status: Least Concern.

Figure 8. Shrimp Crayfish, *Faxonius lancifer*.

***Faxonius longidigitus* (Faxon 1898) - Longpincered Crayfish**

The largest crayfish in Arkansas with its distinctive long, slender pinchers occupies rocky tributaries with permanent flow (and silt-free substrates) of the White and Little Red River systems in northern Arkansas. It can be found living in deeper pools beneath and beside large slab boulders. Pflieger (1996) reported a total length of 25.4 cm (10 in) for this crayfish in Table Rock Lake in Missouri. IUCN Red List Status: Least Concern.

***Faxonius macrus* (Williams 1952) - Neosho Midget Crayfish**

This diminutive stream crayfish inhabits fast-flowing clear streams with gravel and rock substrates and shallow burrows in the upper Arkansas River system in northwest Arkansas. It is also often found in shallow burrows or beneath rocks or boulders (Pflieger 1996). IUCN Red List Status: Least Concern.

***Faxonius marchandi* (Hobbs 1948) - Mammoth Spring Crayfish**

In Arkansas, this uncommon stream crayfish occupies clear streams with riffles and runs, with gravel or rubble substrate of the Spring River drainage in Fulton, Lawrence, Randolph, and Sharp counties. It is also found in high numbers in pools and spring-fed streams (Dukat and Magoulick 1999). In other parts of its range, *F. marchandi* is found in higher numbers in non-permanent freshwater habitats than it is in those which are permanent (Flinders and Magoulick 2003). This species is currently under threat by an invading *F. neglectus chaenodactylus* and ecological impacts on their native range as detailed by DiStefano et al. (2017). The species is currently under consideration for federal endangered species status and would benefit from research to examine gene flow, phylogeographic patterns, and population structure (DiStefano et al. 2017). IUCN Red List Status: Near Threatened.

***Faxonius meeki brevis* (Williams 1952) - Meek's Short Painted Crayfish**

Another rocky stream inhabitant, this form is only found in tributaries of the Arkansas River in extreme northwest Arkansas. This species is found under rocks and is usually associated with rapids. This species is additionally found under debris or in burrows under rocks (Williams 1954). IUCN Red List Status: Least Concern.

***Faxonius meeki meeki* (Faxon 1898) - Meek's Crayfish**

Faxonius meeki meeki is a very common stream crayfish of the Arkansas and White River systems north of 35th parallel in the state (Hobbs 1989). It occupies riffles as well as pool regions where it tends to be found under shelter such as rocks and/or large submerged logs. IUCN Red List Status: Least Concern.

***Faxonius menae* Creaser 1933 - Mena Crayfish**

Robison et al. (2009) described the habitat of *F. menae* as shallow pool margins and shallow runs of clear streams (stream order 1 to 3) under rocks and rubble. In Arkansas, this crayfish is found only in the Ouachita Mountains physiographic province in tributaries of the upper Ouachita River in Hot Spring, Montgomery, and Polk counties (Robison et al. 2009). IUCN Red List Status: Least Concern.

***Faxonius nana* (Williams 1952) - Midget Crayfish**

A small crayfish, *F. nana* inhabits rocky streams of northwestern Arkansas (Benton and Washington counties) in the Neosho River basin. In addition, *F. nana* has been reported in the Illinois River (Bergey et al. 2005), and into the White River drainage (Prairie Creek) of Arkansas (C. Taylor, *pers. comm.*). It is found in clear, flowing permanent streams with substrates consisting of limestone gravel and cobbles (Williams 1952). This species' habitat is under constant threat from agriculture, road construction and urbanization, causing sedimentation and water pollution, in addition to construction of dams. IUCN Red List Status: Least Concern.

***Faxonius neglectus chaenodactylus* (Williams 1952) - Gap Ringed Crayfish**

This form of *F. neglectus* is an uncommon and

poorly-known crayfish (Wagner et al. 2010b). It inhabits streams of the North Fork of the White River and Sylamore Creek in Stone County, and has also been reported from the Spring River basin but is suspected to be an introduction (Rabalais and Magoulick 2006) and potentially invading (DiStefano et al. 2017). IUCN Red List Status: Least Concern.

***Faxonius neglectus neglectus* (Faxon 1885) - Ringed Crayfish**

This invasive crayfish in found in clear, rocky, permanently-flowing streams of the White River (except North Fork) and the Arkansas River system in Arkansas. It can be found in riffles and shallow pools with current. This species is a generalist (Pflieger 1996). IUCN Red List Status: Least Concern.

***Faxonius ozarkae* (Williams 1952) - Ozark Crayfish**

This stream form can be found in the White and Black river systems in northern Arkansas. It occurs in burrows beneath rocks and boulders in silt-free substrates in streams and can also be found in pools and riffles. In addition, *F. ozarkae* is able to survive in dry stream beds in moist burrows (Williams 1954; Pflieger 1996; Flinders and Magoulick 2007). IUCN Red List Status: Least Concern.

***Faxonius palmeri longimanus* (Faxon 1898) - Western Painted Crayfish**

This crayfish is a common and widespread subspecies of streams of the Arkansas River and Red River drainages and upper Ouachita River system from southwestern Arkansas to the mid-central part of the state where it intergrades with the nominate form, *F. p. palmeri*. Its habitat is described as flowing stream reaches with rocky substrate but the species is also found in intermittent pools (Metcalf and Distler 1963). Interestingly, this is the most abundant crayfish inhabiting streams of the Ouachita National Forest of Arkansas. IUCN Red List Status: Least Concern.

***Faxonius palmeri palmeri* (Faxon 1884) - Gray - Speckled Painted Crayfish**

In Arkansas, the nominate form is a stream crayfish occupying northeastern and northcentral Arkansas where it intergrades with *F. p. longimanus* throughout central Arkansas. This subspecies is strictly confined to flowing waters in ditches and streams (Pflieger 1996). Intergrades of *F. palmeri* × *longimanus* were reported from Jackson and Lawrence counties, Arkansas, by Tumlison et al. (2017). IUCN Red List Status: Least Concern.

***Faxonius punctimanus* Creaser 1933 - Spothanded Crayfish**

Recently, McAllister and Robison (2012) reviewed the distribution, life history and conservation status of *F. punctimanus* in northern Arkansas. In southern Missouri, Pflieger (1996) reported this species was abundant in protected areas along the shore where there was cover in the form of vegetation, detritus, or large rocks. McAllister and Robison (2012) documented this crayfish was always found in clear, gravel-bottomed pool areas and only occasionally in swift riffles. It was most often hiding beside rocks and debris or under rocks in the pool regions of the stream, but patrolled pool bottoms regularly. They reported *F. punctimanus* from the White River system in Baxter, Clay, Fulton, Independence, Izard, Lawrence, Marion, Randolph, Searcy, Sharp, and Stone counties. IUCN Red List Status: Least Concern.

***Faxonius virilis* (Hagen 1870) - Virile Crayfish**

This wide-ranging lentic and lotic species is sporadically observed in Arkansas. This crayfish is commonly found on rocky substrates; however, in slower rivers, it is found on a variety of material such as mud, silt, and sand. Occasionally, *F. virilis* constructs burrows in river banks, which have been found to occur at up to 10 m deep (Taylor and Schuster 2004). It is a variable species in need of taxonomic study across its range as there are probably several species masquerading as *F. virilis* currently across the United States. IUCN Red List Status: Least Concern.

***Faxonius williamsi* (Fitzpatrick 1966) - Williams' Crayfish**

Faxonius williamsi is a tertiary burrower occupying cavities excavated under rocks seated in gravel in upland streams (Pflieger 1996). Fitzpatrick (1966) found it in pool regions, but was replaced by *F. m. meeki* in riffles. Our research has shown this species to be a pool inhabitant living in burrows or excavations under rocks in upland clear streams. Robison (1997) also found it living at the base of a waterfall in a shallow pool with rubble and cobble substrate in Washita Creek, Franklin County (Arkansas River drainage). The distribution of *F. williamsi* in Arkansas is the headwaters of the White River in Benton, Boone, Carroll, Madison, and Washington counties and in the Arkansas River drainage in Franklin and Johnson counties. Robison (1997) initially reported *F. williamsi* in the Arkansas River Drainage tributary of Walnut Creek in Johnson County, but was not cited by Wagner et al. (2010a). Taylor et al. (2007) provided a status of

"currently stable" for *F. williamsi* based on long-term research on Arkansas crayfishes by Robison (1997) and Westhoff et al. (2006). IUCN Red List Status: Least Concern.

GENUS *PROCAMBARUS* ORTMANN 1905

***Procambarus acutus* (Girard 1852) - White River Crayfish**

Procambarus acutus (Fig. 9) occupies permanent sluggish to moderately flowing streams and other lentic habitats (Hobbs 1989) where it is commonly collected on the Coastal Plain. In Missouri, 70% of occurrences of *P. acutus* were from standing-water habitats, with the remainder from ditches and small to medium-sized streams (Pflieger 1996). It is known to burrow to avoid drying conditions and spends the winter months in burrows. This crayfish is used in both aquaculture and for fishing bait. IUCN Red List Status: Least Concern.

Figure 9. White River Crayfish, *Procambarus acutus.*

***Procambarus clarkii* (Girard 1852) - Red Swamp Crayfish**

This tertiary burrower occupies lentic and lotic habitats but can be found in burrows (Hobbs 1989) on the Mississippi Alluvial Plain in eastern Arkansas. This crayfish is commonly raised by commercial crayfish producers in the eastern portion of the state for human consumption and has become a serious introduced agricultural pest (Huner 1977). IUCN Red List Status: Least Concern.

***Procambarus curdi* Reimer 1975 - Red River Burrowing Crayfish**.

This species is a primary burrower and an inhabitant of lentic and sluggish lotic habitats in the Red River basin of southwestern Arkansas in Little River, Howard, and Miller counties, and adjacent southeastern Oklahoma (McAllister et al. 2011b). It burrows in sandy soil but can inhabit much harsher environments (Hobbs 1989). IUCN Red List Status: Least Concern.

***Procambarus dupratzi* Penn 1953 - Southwestern Creek Crayfish**

A stream form, *P. dupratzi* occupies the Red River system of southern Arkansas. Walls and Black (2008) suggested that records of *P. dupratzi* from Arkansas and Oklahoma refer to an undescribed species. Molecular analysis will be necessary to confirm this report. IUCN Red List Status: Least Concern.

***Procambarus elegans* Hobbs 1969 - Elegant Creek Crayfish**

This larger member of the distinctive *Pennides* group can be occasionally encountered in permanent streams of the lower Ouachita River system in southern Arkansas. It is found in streams with brown water that flows from sluggish to moderately swift through multiple channels in an eroded clay substrate (Hobbs 1969). IUCN Red List Status: Data Deficient.

***Procambarus geminus* Hobbs 1975 - Twin Crayfish**

Hobbs (1975) described this inhabitant and close relative of *P. acutus* from lentic and lotic habitats of the Red River basin in Columbia, Lafayette, and Miller counties. It occurs in muddy sloughs, ditches, and muddy streams (Walls 2009). Tumlison et al. (2017) documented an ovigerous *P. geminus* from the state as well as new collections from Columbia and Lafayette counties. The type locality is located near Taylor, Columbia County (Hobbs 1975). IUCN Red List Status: Least Concern.

***Procambarus liberorum* Fitzpatrick 1978 - Osage Burrowing Crayfish**

This primary burrower appears to have originated in the White River headwaters of the Ozark Mountains, migrated southward through the Arkansas River drainage onto the north flank of the Ouachita Mountains, then proceeded eastward through the Arkansas River Valley as far east as Lonoke County in the Gulf Coastal Plain province (Crandall et al. 2009). The overall range of *P. liberorum* in Arkansas includes 18 counties, namely Benton, Conway, Crawford, Faulkner, Franklin, Johnson, Logan, Lonoke, Madison, Montgomery, Perry, Polk, Pope, Pulaski, Scott, Sebastian, Washington, and Yell (McAllister et al. 2011b). IUCN Red List Status: Least Concern.

***Procambarus natchitochae* Penn 1953 - Red River Creek Crayfish**

This crayfish is a creek and stream inhabitant of

tributaries of the Red River drainage in southwestern Arkansas. It inhabits clear to slightly cloudy waters with a moderate current and sandy and rocky substrate, as well as pools and roadside ditches (Hobbs 1989). McAllister (*unpubl.*) has found *P. natchitochae* to be very common in similar waters at Nix Creek in Texarkana, Miller County. IUCN Red List Status: Least Concern.

***Procambarus ouachitae* Penn 1956 - Ouachita River Crayfish**

This crayfish is found in 11 counties of the Ouachita and Arkansas River systems, and is a stream form commonly encountered in southcentral and western Arkansas. Tumlison and Robison (2010) added a new county record for *P. ouachitae* in Bradley County. We (CTM and HWR) have found this crayfish inhabiting a spring site (Abernathy Spring) in Polk County (Ouachita drainage). IUCN Red List Status: Least Concern.

***Procambarus parasimulans* Hobbs and Robison 1982 - Bismarck Crayfish**

Another Arkansas endemic, *P. parasimulans* inhabits burrows in lentic and sluggish lotic situations (Hobbs and Robison 1982). This secondary burrower has been documented by HWR in collections from the Arkansas, Ouachita, and Red River basins in southwestern Arkansas in Clark, Grant, Hot Spring, Nevada, Ouachita, Pike and Sevier counties (Hobbs and Robison 1988). IUCN Red List Status: Least Concern.

***Procambarus regalis* Hobbs and Robison 1988 - Regal Burrowing Crayfish**

This is a state endemic primary burrower found in the southwestern corner of Arkansas in the Red River drainage of Howard, Nevada, and Sevier counties (Hobbs and Robison 1988). This species is found in simple burrows and temporary pools (Hobbs and Robison 1988). IUCN Red List Status: Data Deficient.

***Procambarus reimeri* Hobbs 1979 - Irons Fork Burrowing Crayfish**

This state endemic inhabits burrows and temporary pools in the upper Ouachita River basin (Upper Irons Fork) in Polk County in westcentral Arkansas (Robison and Allen 1995). It is known from only 6 localities (Hobbs and Robison 1988). IUCN Red List Status: Least Concern.

***Procambarus simulans* (Faxon 1884) - Southern Plains Crayfish**

A secondary burrower, *P. simulans* is rarely collected in Arkansas. It has been found in lentic and lotic habitats and burrows in the southwestern part of the state in Sevier County (Hobbs and Robison 1988). IUCN Red List Status: Least Concern.

***Procambarus tulanei* Penn 1953 - Giant Bearded Crayfish**

This more widespread secondary burrower has been captured in lentic and lotic habitats and burrows in the Arkansas, Ouachita, and Red River basins in Ashley, Columbia, Drew, Hot Spring, Lafayette, Montgomery, Nevada, Ouachita, and Union counties of the state (Hobbs and Robison 1988). Additional collection/new county records for *P. tulanei* were provided by Tumlison and Robison (2010) in Bradley, Clark, and Union counties. Mature specimens live in simple burrows often capped with large chimneys 30 cm (12 in) high (Walls 2009). IUCN Red List Status: Least Concern.

***Procambarus viaeviridis* (Faxon 1914) - Vernal Crayfish**

Procambarus viaeviridis is taken from sluggish streams and lentic situations on the Mississippi Alluvial Plain of eastern Arkansas. The type locality is the St. Francis River, Clay County (Faxon 1914). IUCN Red List Status: Least Concern.

***Procambarus vioscai vioscai* Penn 1946 - Percy's Creek Crayfish**

In Arkansas, this stream crayfish inhabits tributaries of the Red River system in the southern part of the state. This species can be found in waters with sandy silt or gravel substrates. IUCN Red List Status: Least Concern.

Problematic Species in Arkansas

Several species have earlier been erroneously included in the Arkansas crayfish fauna. The Golden Crayfish (*Faxonius luteus*) has been formerly included as occurring in Arkansas based on records from Carroll (White River, Eureka Springs) and Lawrence (Black River, Black Rock) counties; however, Williams (1954) doubted the validity of the White River locality since he was not able to find *F. luteus* in that area. An established population of this species at the Black Rock locality also seems to be questionable, since *F. luteus* is an upland species, and Black Rock lies at the western edge of the Gulf Coastal Plain. Because this location is also considerably downstream from any known population, it seems unlikely that even waifs would occur there (Bouchard and Robison 1980). Thus, we doubt the

presence of this species in Arkansas based solely on these questionable records and have omitted it from our state checklist.

Another species in question is the Water Nymph Crayfish (*Faxonius nais*). Previously, Williams (1954) identified populations in his study as *Orconectes nais*; however, most of these are referable to *F. virilis* (Bouchard and Robison 1980). Williams did not collect live, adult, or reproductive male specimens of *F. nais* with their distinctive color pattern common to members of the Palmeri Group. The different color patterns of *F. nais* and *F. virilis* certainly would have alerted him that the 2 were morphologically very similar, but separate species were present. Until a confirmed population of *F. nais* is found in Arkansas, it is not currently included as part of the Arkansas crayfish fauna. More complete DNA studies of specimens may reveal more regarding this species in the future.

Previously, the Western Plains Crayfish (*Faxonius causeyi*) had been recorded from Arkansas by Reimer (1966). He considered *F. causeyi* to be distinct from its closest ally, *F. virilis*, although he noted that it may only be a subspecies of *F. virilis*. Hobbs (1972b) later regarded *F. causeyi* as a synonym of *F. virilis*. Hobbs (1974) included *F. causeyi* in his checklist, again questioning its taxonomic validity, but retaining the name until a thorough study of it and *F. virilis* is undertaken. We follow Hobbs (1972b) in regarding *F. causeyi* as a synonym of *F. virilis*, but as noted under the *F. virilis* record and by Hobbs, this species complex needs a thorough study.

Walls (2009) suggested that the Marsh Crayfish (*Procambarus hinei*) is likely to be found in southern Arkansas since it occurs in Ouachita Parish, Louisiana, just below the Arkansas border. However, to date, none have been collected in Arkansas.

The Caddo Chimney Crayfish (*Procambarus machardyi*) is another possible addition to future lists. Walls (2009) reported that it was possible that specimens of this species from both Arkansas and Texas may be misidentifications of either *P. curdi* or *P. parasimulans*. However, additional studies will be necessary to confirm this suggestion.

One possible introduction into the state is the Southern White River Crayfish (*Procambarus zonangulus*). Walls (2009) reported that its natural distribution may have been modified by movement for economic purposes because many commercial ponds in central Louisiana (as well as Arkansas and Mississippi) are stocked with a mixture of wild *P. clarkii* and *P. zonangulus* from southern Louisiana. So, the range possibly extends up the Red and Ouachita as well as Mississippi rivers into Arkansas and Oklahoma.

The most recent crayfish to be added erroneously is the former Lonoke Crayfish, *P. ferrugenius* (Hobbs and Robison 1988) which was later determined to be a synonym of *P. liberorum* by Crandall et al. (2009) and thus, was deleted from the state checklist. While we have herein deleted those species not considered a part of the state crayfish fauna, there are crayfish species which will ultimately be added to our state biodiversity. In our studies of state crayfishes, 3 undescribed crayfish species of the genus *Procambarus* have been discovered using genetic analyses from molecular work (Crandall et al. 2009). Formal descriptions of these undescribed forms are currently being prepared. Collecting continues in Arkansas and undiscovered cryptic species may still yet occur in the state.

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APPENDIX. Checklist of the crayfishes of Arkansas.

FAMILY CAMBARIDAE HOBBS 1942

GENUS *BOUCHARDINA* HOBBS 1977

Bouchardina robisoni Hobbs 1977 - Bayou Bodcau Crayfish

GENUS *CAMBARELLUS* ORTMANN 1905

Cambarellus puer Hobbs 1945 - Swamp Dwarf Crayfish
C. shufeldtii (Faxon 1884) - Cajun Dwarf Crayfish

GENUS *CAMBARUS* ERICHSON 1846

Cambarus aculabrum Hobbs and Brown 1987 - Benton County Cave Crayfish
C. causeyi Reimer 1966 - Boston Mountains Crayfish
C. diogenes Girard 1852 - Devil Crawfish
C. hubbsi Creaser 1931 – Hubbs' Crayfish
C. ludovicianus Faxon 1884 - Painted Devil Crayfish
C. setosus Faxon and Garman in Garman1889 - Bristly Cave Crayfish
C. zophonastes Hobbs and Bedinger 1964 - Hell Creek Cave Crayfish

GENUS *CREASERINUS* HOBBS 1973

Creaserinus caesius (Hobbs 1975) - Timberlands Burrowing Crayfish
C. fodiens (Cottle 1863) - Digger Crayfish
C. gilpini (Hobbs and Robison 1989) - Jefferson County Crayfish

GENUS *FALLICAMBARUS* HOBBS 1969

Fallicambarus dissitus (Penn 1955) - Pine Hills Digger
F. harpi Hobbs and Robison 1985 - Ouachita Burrowing Crayfish
F. jeanae Hobbs 1973 - Daisy Burrowing Crayfish Burrowing Crayfish
F. petilicarpus Hobbs and Robison 1989 - Slenderwrist Burrowing Crayfish
F. schusteri Taylor and Robison 2016 - Carmel Burrowing Crayfish
F. strawni (Reimer 1966) - Saline Burrowing Crayfish
F. tenuis (Hobbs 1950) - Ouachita Mountain Crayfish

GENUS *FAXONELLA* CREASER 1933

Faxonella blairi Hayes and Reimer 1977 - Blair's Fencing Crayfish
F. clypeata (Hay 1899) - Ditch Fencing Crayfish

GENUS *FAXONIUS* ORTMANN 1905

Faxonius acares (Fitzpatrick 1965) - Redspotted Stream Crayfish
F. cyanodigitus (Johnson 2010) - Red River Painted Crayfish
F. difficilis (Faxon 1898) - Painted Crayfish
F. eupunctus (Williams 1952) - Coldwater Crayfish
F. lancifer (Hagen 1870) - Shrimp Crayfish
F. leptogonopodus (Hobbs 1948) - Little River Creek Crayfish
F. longidigitus (Faxon 1898) - Longpincered Crayfish
F. macrus (Williams 1952) - Neosho Midget Crayfish
F. marchandi (Hobbs 1948) - Mammoth Spring Crayfish
F. meeki brevis (Williams 1952) - Meek's Short Painted Crayfish
F. meeki meeki (Faxon 1898) - Meek's Crayfish
F. menae Creaser 1933 - Mena Crayfish
F. nana (Williams 1952) - Midget Crayfish
F. neglectus chaenodactylus (Williams 1952) - Gap Ringed Crayfish
F. n. neglectus (Faxon 1885) - Ringed Crayfish
F. ozarkae (Williams 1952) - Ozark Crayfish
F. palmeri longimanus (Faxon 1898) - Western Painted Crayfish
F. p. palmeri (Faxon 1884) – Gray-Speckled Painted Crayfish
F. punctimanus Creaser 1933 - Spothanded Crayfish
F. virilis (Hagen 1870) - Virile Crayfish
F. williamsi (Fitzpatrick 1966) – Williams' Crayfish

GENUS *PROCAMBARUS* ORTMANN 1905

Procambarus acutus (Girard 1852) - White River Crayfish
P. clarkii (Girard 1852) - Red Swamp Crayfish
P. curdi Reimer 1975 - Red River Burrowing Crayfish
P. dupratzi Penn 1953 - Southwestern Creek Crayfish
P. elegans Hobbs 1969 - Elegant Creek Crayfish
P. geminus Hobbs 1975 - Twin Crayfish
P. liberorum Fitzpatrick 1978 - Osage Burrowing Crayfish
P. natchitochae Penn 1953 - Red River Crayfish
P. ouachitae Penn 1956 - Ouachita River Crayfish
P. parasimulans Hobbs and Robison 1982 - Bismarck Crayfish
P. regalis Hobbs and Robison 1988 - Regal Burrowing Crayfish
P. reimeri Hobbs 1979 - Irons Fork Burrowing Crayfish
P. simulans (Faxon 1884) - Southern Plains Crayfish
P. tulanei Penn 1953 - Giant Bearded Crayfish
P. viaeviridis (Faxon 1914) - Vernal Crayfish
P. vioscai vioscai Penn 1946 - Percy's Creek Crayfish

Histology of Rathke's Glands in the Razor-backed Musk Turtle, *Sternotherus carinatus* (Chelonia: Kinosternidae), with Comments on Lamellar Bodies

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Running Title: Rathke's Glands in the Razor-backed Musk Turtle, *Sternotherus carinatus* (Chelonia: Kinosternidae)

Abstract

I examined the histology and ultrastructure of Rathke's glands in two adult male razor-backed musk turtles (*Sternotherus carinatus*) collected in northeastern Arkansas. This species possesses two pairs of Rathke's glands that are embedded beneath marginal bones and are named according to their anatomical location (i.e., axillary and inguinal). These integumentary glands are similar anatomically to one another. Each gland is comprised of a single, highly vascularized secretory lobule, which is surrounded by a thin tunic of asymmetrically arranged, striated muscle. Two types of large secretory vacuoles characterize most of the holocrine cells produced by a relatively thin secretory epithelium. My results suggest that the chief secretory material of the smaller dark-staining secretory vacuole is a glycoprotein complex. The larger, mostly translucent secretory vacuole contains variously sized, multilaminar, osmophilic lamellar bodies, whose structural design is reminiscent of an epidermal lipid delivery system in vertebrates. The function of Rathke's glands in turtles remains unknown.

Introduction

Trauth and Plummer (2013) reviewed the literature on turtle Rathke's glands, which occur in members of 13 of the 14 living chelonian families (Waagen 1972; Ehrenfeld and Ehrenfeld 1973; Solomon 1984; Plummer and Trauth 2009; Trauth 2012). These exocrine integumentary glands, also known as musk or scent glands, number from one to five pairs (Waagen 1972) and release a musty, sometimes-malodorous secretion through external epidermal pores. The glands are named based upon the general location of their orifices (axillary and inguinal) and/or the proximity of the orifices to scutes (e.g., inframarginal). Most Rathke's glands consist of one or more lobules encased within a striated muscle tunic, and the secretory epithelium consists of ovoid-to-spherical holocrine cells (Ehrenfeld and Ehrenfeld 1973; Solomon 1984; Plummer and Trauth 2009; Trauth 2012; Trauth and Plummer 2013). Seifert et al. (1994) and Weldon et al. (2008) reported that the secretions released by these cells are primarily proteins and, to a lesser extent, lipids, as well as various acids. Lamellar bodies may also be present within the secretory vacuoles of these cells (Plummer and Trauth 2009; Trauth 2012; Trauth and Plummer 2013). The function of Rathke's gland secretions remains poorly understood. Few studies have focused on the histology and/or ultrastructure of Rathke's glands in chelonians (Stromsten 1917; Zangerl 1941; Ehrenfeld and Ehrenfeld 1973; Solomon 1984; Weldon and Tanner 1990; Weldon et al 1990; Rostal et al 1991; Plummer and Trauth 2009; Trauth 2012; Trauth and Plummer 2013).

Lamellar bodies are intracellular tubulo-vesicular organelles composed of concentric phospholipid bilayers. These osmophilic structures occur in epithelial cells (e.g., type II alveolar cells, corneocytes, and mesothelial cells) in humans (Schmitz and Müller 1991; Fartasch 2004; Kennish and Reidenberg 2005; Spener et al. 2006; Sato and Ghazizadeh 2009; Vanhecke et al. 2010) and in Rathke's glands of turtles (Ehrenfeld and Ehrenfeld 1973; Maltoltsy and Bednarz 1975, Alibardi and Toni 2006, Plummer and Trauth 2009; Trauth 2012; Trauth and Plummer 2013). Lipid storage and secretion are presumably the primary roles of lamellar bodies. Moreover, the functional characterization of lamellar bodies is mostly restricted to descriptive studies using electron microscopy (Spener et al. 2006). Plummer and Trauth (2009), Trauth (2012), and Trauth and Plummer (2013) illustrated the multilaminar structure of lamellar bodies in turtles using transmission electron microscopy. Other than a study by Mahmoud and Alkindi (2008), which showed the ultrastructure of a lipoidal body within the corpus luteum of the snapping turtle, no additional ultrastructural studies have depicted lamellar bodies in turtles.

My objectives in the present study were to examine the histology and ultrastructure of Rathke's glands in the

adult male razor-backed musk turtle (*Sternotherus carinatus*) and report on the presence of lamellar bodies.

Materials and Methods

I prepared the Rathke's glands from two adult male razor-backed musk turtles collected from northeastern Arkansas (one on 2 March 2012 and the other on 4 April 2016) for light microscopy (LM-plastic) and transmission electron microscopy (TEM) in the lab at Arkansas State University. These two voucher specimens were deposited in the Arkansas State University herpetological collection (ASUMZ 31996 and 33475, respectively). Carapace (CL) and plastron (PL) lengths were measured prior to sacrificing with an intra-pleuroperitoneal injection of sodium pentobarbital following the university's established IACUC protocol for reptile euthanasia.

A Dremel Multi-Max™ oscillating tool was used to extract Rathke's glands from beneath the turtle carapace (Fig. 1). Glands were immediately placed into vials of 2% glutaraldehyde (GTA) solution buffered with 0.1 M sodium cacodylate at a pH of 7.2 and allowed to fix for 2 h. For postfixation, I used 1% w/v osmium tetroxide, buffered as above, for 2 h. I have previously described the methods used to prepare tissues for LM-plastic (Trauth 2012). In brief, I dehydrated glands in 20 min increments into increasing concentrations of ethanol (70-100%) and then placed the glands in a 50/50% acetone/plastic mixture for overnight infiltration via rotation. For thick sectioning (approximately 1 µm in thickness) and staining, I used glass knives on an LKB Ultrotome (Type 4801A) with Ladd® multiple stain (LMS), respectively. For photomicroscopy, I used a Nikon Eclipse 600 epi-fluorescent light microscope with a Nikon DXM 1200C digital camera (Nikon Instruments Inc, Melville, NY). A Canon T4i digital single lens reflex camera fitted with a macro lens was also used to photograph macroscopic images of the turtle carapace and internal glands.

Plastic-embedded samples prepared for light microscopy were also utilized for TEM. Trimmed tissue blocks were sectioned on a diamond knife. Sections were picked up with 150 - 200 mesh copper grids, stained with uranyl acetate (3% aqueous) and lead citrate for 30 min each. Grids were examined with a JEOL 100 CX-II transmission electron microscope (JEOL USA, Inc., St. Louis, MO) at 60 kV (55 µA). Positive digital images were generated by scanning developed TEM negatives using an Epson Perfection 4990 scanner (Epson America, Inc., Long Beach, CA).

I followed the descriptive terminology for Rathke's glands used by Ehrenfeld and Ehrenfeld (1973), Solomon (1984), Plummer and Trauth (2009), Trauth (2012), and Trauth and Plummer (2013). In addition, the descriptive ultrastructure for lamellar bodies followed previous investigations on snapping turtles and hatchling three-toed box turtles (Trauth 2012; Trauth and Plummer 2013).

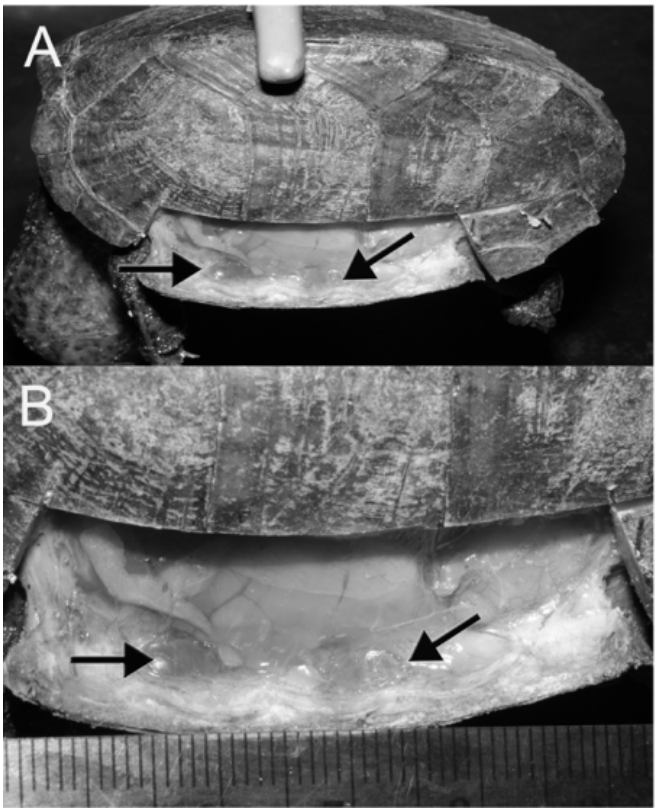

Figure 1. Rathke's glands in a small adult male *Sternotherus carinatus* (ASUMZ 31996; CL = 88 mm). A. Dissection of glands begins with the dorsal and lateral intrusion into carapace and marginal regions. B. Left arrow points to the left axillary gland and right arrow to the left inguinal gland (metric scale in mm).

Results

Gross Morphology

From a dorsal perspective, the axillary pair of Rathke's glands lie beneath the posterolateral edge of costal scute 1 and extend into the anterior portion of costal scute 2 (Fig. 1A and B). The inguinal glands are situated beneath the anteriolateral edge of costal scute 3 (Fig. 1.) Internally, the glands are positioned within slight depressions of the interior marginal bones. The glands' dimensions are variable according to turtle body size, but fall between 6 - 10 mm in length and 4 - 6 mm in width.

Light Microscopy

Both the axillary and inguinal glands in *S. carinatus* are comprised of an elongated, circular lobule, whose lumen is filled with opaque secretory material (Fig. 2A and B) and/or secretory vacuoles. The secretory epithelium rests upon a thin basement membrane (Fig. 3B). A thin-to-moderately thick layer of dense connective tissue is contiguous with the basal lamina. In general, the secretory epithelium is comprised of a thin, basal, generative single cell layer of holocrine cells (Fig. 3). These epithelial cells proliferate outward into an expansive lumen (Fig. 2). The external wall of each gland is made of a uniformly thick muscular tunic (Fig. 3B). At some point following their release from the apical region of the secretory epithelial cell surface, secretory cells lose their structural integrity and degenerate, dumping their cellular contents into the glandular lumen. Eventually, a flocculent conglomerate (a more or less homogenous cellular fluid and debris mixture) becomes the material that is eventually passed into an excretory duct that leads to the exterior.

Two different types of secretory vacuoles (Type 1 and Type 2) were observed in the secretory epithelium in all Rathke's glands (Figs. 2 and 3). Type 1 secretory vacuoles are generally smaller than Type 2 and normally appear as single, dark-staining spherical or oval masses (Fig. 3). Their matrix is not removed during tissue preparation. In contrast, Type 2 secretory vacuoles are large circular-to-oblong organelles, when fully distended, and generally appear mostly devoid of material. These vacuoles are normally referred to as lipid droplets. Irregularly shaped osmophilic, lipoidal membrane-bound structures are clustered unevenly within Type 2 secretory vacuoles. Soluble lipids found in these lipoid droplets are removed from these vacuoles during histological preparation (Fig. 4).

Transmission Electron Microscopy

The ultrastructure of lamellar bodies of Rathke's glands is shown in Figure 4. Individual lamellar bodies may exhibit numerous bilayered membranes that may surround an electron-dense core region (Fig. 4C). Lamellar bodies are conspicuous dark entities observed in Type 2 secretory vacuoles when viewed with light microscopy (Fig. 3). The arrangement of the circular lamellar bodies varied, but they were observed scattered along the distal inner membrane surface of the Type 2 secretory vacuole (faintly apparent in vacuoles shown in Fig. 3A and C).

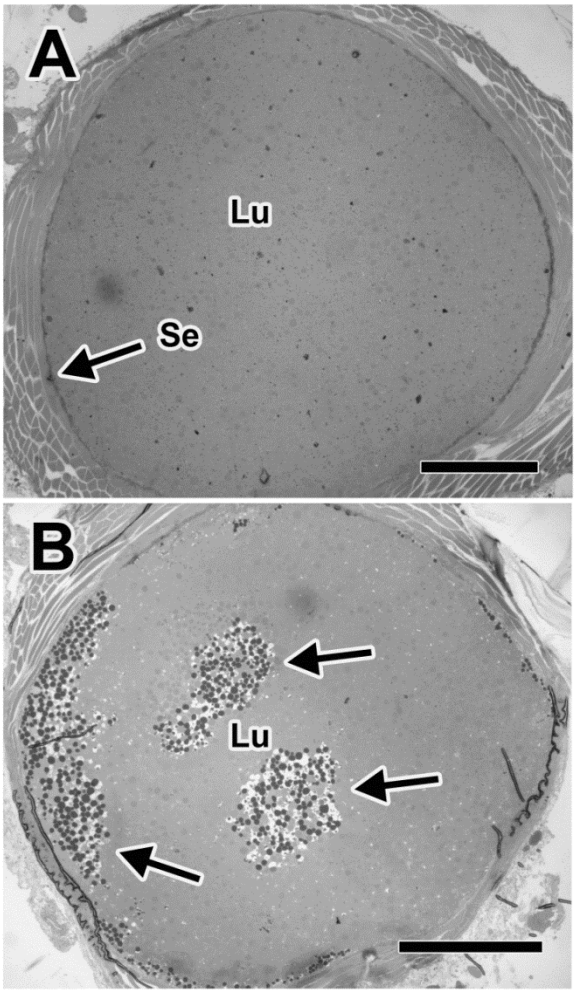

Figure 2. Light micrograph of left axillary (A) and right inguinal (B) Rathke's gland in *Sternotherus carinatus* (ASUMZ 31996). A. Transverse section through gland lumen (Lu) filled with opaque secretory material. Secretory epithelium (Se) exhibits few secretory vacuoles. B. Transverse section through gland lumen (Lu) exhibiting clusters of secretory vacuoles (ends of arrows). Scale bar = 50 μm for A and B.

Discussion

Rathke's glands of relatively few non-marine turtles have been studied anatomically or histologically in any detail; however, a number of common morphological and histological features occur among those species. For example, the glands of *Sternotherus odoratus* (Ehrenfeld and Ehrenfeld 1973), *Apalone mutica* and *A. spinifera* (Plummer and Trauth 2009), *Kinosternon subrubrum* (Webb 2010), and *Terrapene carolina* and *T. ornata* (Trauth and Plummer 2013) are comprised of either a single lobule or, in other cases, multiple lobules, which exhibit a thin to relatively thick layer of loose connective tissue immediately surrounding the secretory

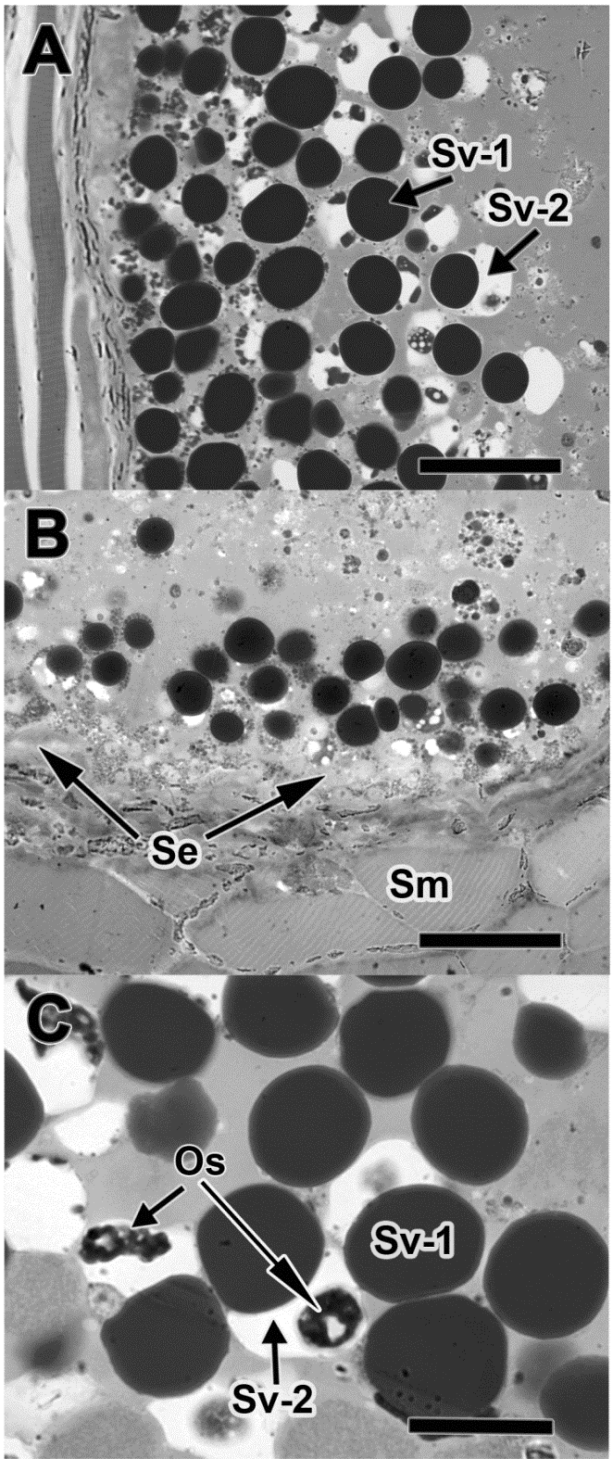

Figure 3. Light micrographs of axillary glands in *Sternotherus carinatus* (A and C, ASUMZ 31996; B, ASUMZ 33475).
A. Section showing secretory epithelium with numerous holocrine cells containing smaller, dark-staining, Type 1 secretory vacuoles (Sv-1) and larger, lipid droplets (clear spheres) characteristic of Type 2 secretory vacuoles (Sv-2). B. Section similar to A. C. Magnification of Type 1 and 2 secretory vacuoles; some Type 2 vacuoles contain osmophilic lamellar bodies (Os). Se = secretory epithelium; Sm = striated muscle. Scale bars in A and B = 50 µm; in C = 20 µm.

epithelium. All are also wrapped in a tunic of striated muscle, and all receive a rich supply of blood from capillaries that lie in close proximity to the basal lamina of the secretory epithelium. Despite these structural similarities, hatchling box turtles, for example, possess glands with holocrine cells that more closely resemble those of *Apalone* and *Kinosternon* than to those of *Sternotherus carinatus*. Although all these species studied thus far possess at least two types of epithelial cells (basal and secretory), *Sternotherus odoratus* differs from the others by possessing a third cell, best described as a holocrine cell containing a number of small lipoid droplets (Ehrenheld and Ehrenheld 1973). These lipoid cells are concentrated within the center of the glandular lumen. Trauth and Plummer (2013) identified solitary large Type 1 secretory vacuoles in box turtles, and these secretory vacuoles were also present in softshell turtles (Plummer and Trauth 2009) and in the mud turtle (Webb 2010). The secretory material of Type 1 secretory vacuoles was putatively identified as a glycoprotein complex in *Sternotherus odoratus* as shown by Ehrenheld and Ehrenheld (1973) based upon PAS+ staining results. The carbohydrate component of the glycoprotein comprised less than 4% of the total molecule in *Sternotherus*. We found similar staining results in the holocrine cells of box turtles and razor-backed musk turtles as did Webb (2010) for *Kinosternon subrubrum*.

Type 2 secretory vacuoles of the razor-backed musk turtle were generally large open spheres, which contained lamellar bodies various sizes and shapes. This type of microstructure was also apparent in *Sternotherus odoratus* (Trauth 2012). In general, lamellar bodies are similar to one another in all turtles studied thus far, although the lamellar membranes, for the most part, were more densely compacted and more numerous in both species of *Apalone* (Plummer and Trauth 2009) . Lamellar bodies may play a role in lipid transfer (Ehrenheld and Ehrenheld 1973), but their function remains unknown in Rathke's glands.

Rathke's glands in razor-backed musk turtles normally exude a malodorous substance, as is the case in most turtles. For instance, the foul-smelling secretion may be present in both adult and hatchling *Terrapene* spp. (Neill 1948; Norris and Zweifel 1950; Legler 1960; Patton et al. 2004; Gangloff and Nash 2010). Gangloff and Nash (2010) detected a musk odor in 12 of 34 hatchling *T. ornata* and 2 of 48 adult *T. ornata*. Patton et al. (2004) detected musk odor in 315 of 1407 (22.4%) hatchling *T. carolina*, but did not detect odor in any individuals more than a few days old. Based on the human detection of a musky odor, Patton et al. (2004)

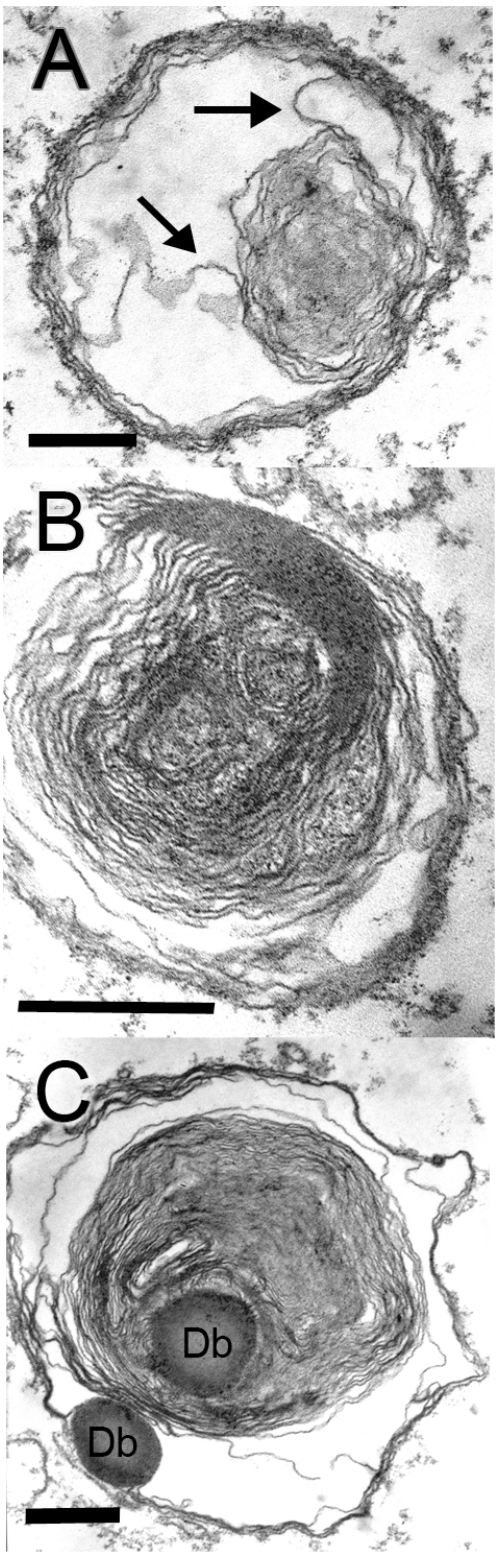

Figure 4. Transmission electron micrographs of lamellar bodies of Rathke's glands in *Sternotherus carinatus* (ASUMZ 31996). A. Lamellar body showing formation of bilayers. (arrows) around an eccentric core. B. Lamellar body showing multilaminar bilayers C. Lamellar body with associated electron dense granules (Db). Scale bars = 0.5 µm for A-C.

concluded that relatively few *T. carolina* possess Rathke's glands at birth and in those that did possess the glands, function decreased with age. The incidence of siblings producing a musk odor within 503 different clutches varied from 4 to 54% (Patton et al. 2004). Corroborating the conclusion, based on behavior, that relatively few individuals possess Rathke's glands at birth, Waagen (1972) found the physical presence of Rathke's glands in only three of 16 (19%) dissected *Terrapene* individuals.

The presence of Rathke's glands is thought to be the basal condition for all turtles (Waagen 1972; Weldon and Gaffney 1998). Their absence is presumably an apomorphic condition. Terrestrial turtles (testudinoids and a few emydids--Ehrenfeld and Ehrenfeld 1973; Waagen 1972) generally lack the glands. Rathke's glands may be of less biological importance in the terrestrial environment.

Acknowledgments

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Distribution, Habitat, and Life History Aspects of the Shrimp Crayfish, *Faxonius lancifer* (Hagen) (Decapoda: Cambaridae) in Arkansas

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Running Title: *Faxonius lancifer* in Arkansas

Abstract

The Shrimp Crayfish, *Faxonius* (formerly *Orconectes*) *lancifer* (Hagen) is an uncommon, although widespread, crayfish in Arkansas. This species is herein documented from 19 counties of the Gulf Coastal Plain physiographic region. Between 1974 and 2017, we made 344 collections throughout the 75 counties of Arkansas, of which 22 (6%) yielded 163 specimens of *F. lancifer*. Thus, from these collections, plus 10 unpublished collections of Reimer (1963), and one collection from G.L. Harp, a total of 34 collections of *F. lancifer* are now known from the state. *Faxonius lancifer* ranged from uncommon (1 specimen) to locally abundant (39 specimens) at these collecting localities. With regard to conservation status, *F. lancifer* should be considered as "Currently Stable" due to its widespread distribution and general abundance in Arkansas.

Introduction

Freshwater crayfish of the family Cambaridae reach their greatest diversity in North America north of Mexico, totaling 374 species with new species described almost yearly (Taylor et al. 2007; Crandall and Buhay 2008). Crayfishes are also important components of the aquatic ecosystem (Huryn and Wallace 1987; Momot 1995; Usio and Townsend 2004).

One of the smaller species of crayfish in Arkansas is the Shrimp Crayfish, *Faxonius* (syn. *Orconectes*) *lancifer* (Hagen). The precise distribution of this species in the state is poorly known and little has been recorded about its natural history, including ecology, reproductive biology, habitat characteristics, and general biology. This study was initiated to learn more about *F. lancifer* and to discern its geographical distribution within Arkansas.

Specific objectives of the study were (1) to determine the relative abundance and precise distributional limits of the range of *F. lancifer* in Arkansas, (2) to gather data on aspects of life history of this crayfish species, including information on habitat, reproductive period, and any other biological data available, (3) to document ecological and habitat characteristics of this crayfish species, and (4) to assess the current conservation status of *F. lancifer* based on the collected distributional data in the state.

Materials and Methods

Field work was conducted between March 1974 and July 2017, with a total of 344 collections made in 75 counties throughout Arkansas. The bulk of the field work occurred during the fall, spring, and summer. Aquatic dipnets, seines, and both baited and unbaited Gee® minnow traps were used to collect *F. lancifer*. Most individuals were released unharmed at the collecting site; voucher specimens were preserved in 60% isopropyl or ethanol. The number of specimens in the Appendix represents the number of specimens preserved (historical data) or the total number collected at an individual site. Preserved vouchers were deposited in the Southern Arkansas University (SAU) Invertebrate Collection, the Illinois Natural History Survey (INHS) crayfish collection, Smithsonian National Museum of Natural History (USNM), and the Brigham Young University (BYU) crayfish collection.

In addition to collections made during this survey, museum specimens housed at the USNM (USNM 2016), INHS (2016), BYU, and SAU were used to document the current distribution of *F. lancifer* in Arkansas. All previous literature dealing with this crayfish species was also consulted. Both our survey and historical collection locations were converted to latitude/longitude for documentation (Appendix).

The crayfish taxa listed herein, including *F. lancifer*, are updated using the classification scheme of Crandall and De Grave (2017) which better reflects

evolutionary associations of crayfish species.

Results and Discussion

Our survey located 163 specimens of *F. lancifer* in 22 of 344 (6%) localities, plus 10 localities from Reimer (1963) and 1 collection of G.L. Harp, all collected from 19 counties of Arkansas (see Appendix, Fig. 1). This crayfish was found in ditches, backwater areas of streams, and lakes.

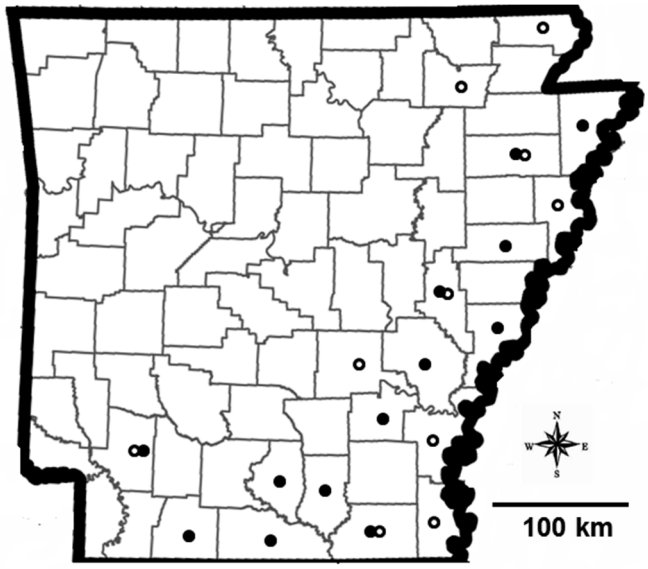

Figure 1. County distribution of *Faxonius lancifer* in Arkansas. Open dots = previous records; solid dots = new records.

Recognition Characters of F. lancifer

Faxonius lancifer is readily recognized in the field by its reddish-brown to gray body coloration thickly dusted with darker specks giving it a somewhat mottled appearance, the short, narrow chelae (dactyl shorter than the length of the palm lacking longitudinal ridges and tubercles), and an acumen that is longer than the rest of the dorsum (Fig. 2). The carapace has strong cervical spines and the carapace and abdomen are about equal in length. The rostrum of *F. lancifer* is wide with a deep, central, trough-like depression and lateral spines and branchiostegal spines are absent. The areola is absent and the antennal scale is widest at the point anterior to mid-length. Adults rarely exceed 76 mm total length (TL) (Morehouse and Tobler 2013). The first pleopod of Form I males terminates in 2 very short processes while the mesial process is noncorneous and equal in length or slightly longer than the central projection. Pflieger (1996, Plate 14) provided line drawings of

Figure 2. The Shrimp Crayfish, *Faxonius lancifer*, from S of Crossett, Ashley County, Arkansas.

Form I and II male gonopods. The annulus ventralis of the female lacks a well-developed fossa (Taylor and Schuster 2004). Hobbs (1989) figured the gonopod, carapace, antennal scale, chela and carpus, and annulus ventralis of *F. lancifer*.

Relative Abundance

It appears that *F. lancifer* ranges from relatively uncommon to locally common in certain parts of Arkansas. Reimer (1963) made 289 collections amassing 7,300 specimens and 33 species in 4 genera in his study of Arkansas crayfishes. In the present study, 163 specimens of *F. lancifer* were taken in 22 of 344 collections (6%) made in Arkansas since 1974. Combining our data with Reimer (1963), and a single collection by G.L. Harp, we determined that, of 633 collections made in Arkansas (between 1963 and 2015), only 226 individuals of *F. lancifer* have been taken from the state. Most of these are housed in museums and a few were also retained at BYU for eventual DNA analyses. Collections at individual sites ranged from 1 specimen to 39 (USNM 146076).

Habitat - Arkansas

In Arkansas, Reimer (1963) noted that specimens of *F. lancifer* were from moderately shallow water, less than 0.61 m (2 ft) deep. The water was usually standing, clear, and void of vegetation. Bottom conditions were mud and clay. Our 42 yrs of collecting in all 75 counties in Arkansas has established *F. lancifer* as an inhabitant of permanent lentic situations in roadside ditches, intermittent first-order streams, sloughs with heavy vegetation, oxbow lakes, edges of swamps, and large river backwaters. Substrates have usually been sand,

mud, and/or clay. Because this species is a burrower, when water levels recede, individuals construct simple burrows 10 to 30 cm deep topped by small chimneys of tiny round pellets. We found for most of the year, as did Pflieger (1996), *F. lancifer* is sequestered in these burrows

Habitat – Louisiana, Missouri, Oklahoma

In Louisiana, Walls (2009) noted that *F. lancifer* was seldom found in permanent waters deep enough for predatory fish, but preferred shallow ditches, sloughs, and ponds with permanent vegetation. Penn (1952) summarized the habitat of *F. lancifer* in Louisiana by saying it occurs most frequently in moderate depth water (i.e., more than 38 cm [15 in] deep), which is clear, permanent, either flowing or static, and exposed to full sunlight. Most of his collections were from habitats with mud or mud and sand bottoms and with little or no aquatic vegetation present. Pflieger (1969) collected this crayfish from small intermittent creeks and the shallows of seasonally flooded sloughs and swamps in Missouri. Interestingly, this species can survive drying conditions by finding refuge under woody debris and thick vegetation patches as it is a tertiary burrower (Pflieger 1996, Taylor and Schuster 2004). In Oklahoma, *F. lancifer* is generally found in swamps, oxbow lakes, and floodplains with mud and silt substrates, but has also been taken in large slow moving rivers (Morehouse and Tobler 2013).

Distribution

The native range of *F. lancifer* includes southwestern Illinois and southeast Missouri to the Lower Mississippi Alluvial Valley and the Gulf Coastal Plain from southeastern Oklahoma, eastern Texas, and Louisiana to Mississippi (Hobbs 1989, Pflieger 1996, Taylor and Schuster 2004, Walls 2009, Morehouse and Tobler 2013); beyond the Mississippi River basin, this crayfish has scattered records across the Coastal Plain in Alabama and Mississippi, especially near the Gulf Coast (Adams et al. 2010, 2015).

Collections of crayfishes have been made in all 75 Arkansas counties by one of us (HWR) during the past 42 yrs. Data from these collections revealed an absence of *F. lancifer* from the Ozark and Ouachita Mountains physiographic regions as well as the Arkansas River Valley. *Faxonius lancifer* occupies the Coastal Plain province in Arkansas, becoming less abundant in northeastern Arkansas and extreme southwestern Arkansas. At most of these locations *F. lancifer* ranged from an uncommon to a locally abundant crayfish.

In an unpublished thesis, Reimer (1963) made 12 collections of *F. lancifer* and documented it from 10 counties in Arkansas including Ashley, Chicot, Clay, Crittenden, Desha, Hempstead, Jefferson, Lawrence, Monroe, and Poinsett. Our studies amassed a total of 20 collections of *F. lancifer* from 9 additional counties of the state including Arkansas, Bradley, Calhoun, Columbia, Lincoln, Mississippi, Phillips, St. Francis, and Union (Appendix). Specific localities for *F. lancifer* (*n* = 163 specimens) are listed in the Appendix. *Faxonius lancifer* was documented from 19 counties throughout the Coastal Plain of Arkansas, and 9 (47%) of them are new county records (Fig. 1). The largest number of specimens collected at one time was 39 individuals (USNM 146076) collected on 16 August 1974 by HWR from Bayou Bartholomew at St. Hwy. 293. Even though this crayfish was collected throughout the Coastal Plain province (Fig. 1), most often *F. lancifer* was found associated with pine woodlands or otherwise forested areas rather than open alluvial farming areas. In Ashley County, we found this crayfish to be common in ruts along a power line bordered by pine woodlands. This finding mirrors what Walls (2009) found in Louisiana as he collected *F. lancifer* mostly in the pinelands, not in the alluvial soils of the Mississippi and Atchafalaya basins.

Life History Aspects – Arkansas

In our study, 5 ovigerous females were collected on 21 February 1984 (3 specimens) and 2 March 1974 (2 specimens) whereas 3 adult females with young attached were collected on 11 April 1991. We found form I males only in August and September. Form II males were collected in July and August. Mature females were taken from June to September. Juveniles were collected in May to July. Adult specimens of *F. lancifer* in the study ranged from 5.8–8.6 cm (2.3–3.4 in) in TL.

Life History Aspects - Illinois, Louisiana, and Missouri

In Louisiana and Illinois (Page 1985, Walls 2009), Form I males have been collected from August to November, which corresponds to the peak of their breeding activities (Black 1972). Form I males (5.3–6.6 cm [2.1–2.6 in]) TL have been collected in September in Missouri (Pflieger 1996). Form II males and females have been taken year round, but dominate collections from April to July. Page (1985) reported ovigerous

females were collected in September and October in Illinois, whereas in Louisiana, ovigerous females and females carrying young have been found in February (Walls 2009). A single female with 570 eggs was reported from Louisiana. Juveniles have been found in Louisiana in late spring into early summer (Walls 2009). In Missouri, 40 juveniles were collected in July ranging from 3.0–4.6 cm (1.2–1.8 in) in TL (Pflieger 1996).

Decapod Associates

Ten crayfish associates were collected throughout the state with *F. lancifer*, including the Digger Crayfish (*Creaserinus fodiens*), Painted Devil Crayfish (*Cambarus ludovicianus*), Devil Crayfish (*C. diogenes*), Swamp Dwarf Crayfish (*Cambarellus puer*), Cajun Dwarf Crayfish (*Ca. shufeldtii*), Ditch Fencing Crayfish (*Faxonella clypeata*), Twin Crayfish (*Procambarus geminus*), White River Crayfish (*P. acutus*), Ouachita River Crayfish (*P. ouachitae*), and Giant Bearded Crayfish (*P. tulanei*). Reimer (1963) reported 2 additional crayfish associates: Western Painted Crayfish (*Faxonius palmeri longimanus*), and Gray-Speckled Crayfish (*F. p. palmeri*).

Conservation Status

Taylor et al. (2007) estimated that 48% of the North American crayfish fauna required some sort of conservation status and protection. They designated *F. lancifer* as a "Currently Stable" (CS) species, defined as a species or subspecies whose distribution is widespread and stable and is not in need of immediate conservation management actions. Our discovery of 164 individuals of *F. lancifer* across 19 counties in Arkansas establishes this crayfish as uncommon in the state; however, we feel more concentrated collecting in southeastern and northeastern Arkansas might yield additional localities and individuals, even though this area has been heavily polluted with herbicides and various insecticides, particularly those targeting cotton- destroying insects. We therefore concur with Taylor et al. (2007) with the CS designation of *F. lancifer* in Arkansas.

In summary, within Arkansas, *F. lancifer* primarily inhabits the Gulf Coastal Plain physiographic province. Our research indicates this species is fairly widespread; however, uncommon in the state. The distributional range of *F. lancifer* includes 19 counties located principally in southeastern and northeastern Arkansas. At each location within these counties, *F. lancifer* ranges from uncommon to locally abundant.

Acknowledgments

HWR especially wants to thank former SAU students who assisted him with field collections of crayfishes (1974 to 2008) including, K. Ball, N. Covington, D. Koym, C. Marsh, and J. Rader. The Arkansas Game and Fish Commission (AG&F) provided scientific collecting permits to the authors. We also thank B.K. Wagner (AG&F) for sharing the database on Arkansas crayfishes.

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Appendix. County locations of 164 specimens of *Faxonius lancifer* from Arkansas (locality, latitude/longitude in decimal degrees or township, section, and range [if known], date of collection, collector(s), museum collection, and number of specimens). HWR = Henry W. Robison; RR = R. Reimer; RT = Renn Tumlison.

Arkansas County (*n* = 1)
(1) Crooked Creek at U.S. Hwy. 79 bridge, ca. 16 km SW of Stuttgart (Bayou Meto Dr.) (34.4265°N, 91.6678°W). 27 September 1974. HWR. USNM 146740 (1 male I).

Ashley County (*n* = 5)
(1) Unnamed creek, 13.7 km W of Snyder (33.2525°N, 91.7381°W). No date. RR. (Reimer 1963). (1).
(2) Roadside ditch, 5 km SE of Hamburg on St. Hwy. 8 (Sec. 28, T17S, R6W). 19 Apr. 1990. (Tumlison and Robison 2010). HWR. BYU (3).
(3) Ruts along a power line S of Crossett near St. Hwy. 133 (33.1031°N, 91.9478°W). 30 Jun. 2014. RT, photovoucher (see Fig. 2). (1).

Bradley County (*n* = 41)
(1) Roadside ditch, 4.5 km E of Banks on St. Hwy. 275. 18 Apr. 1986. HWR. USNM 218922 (31).
(2) Moro Creek at St. Hwy. 160, SE of Harrell. 23 Apr. 2003. HWR. SAU (10).

Calhoun County (*n* = 10)
(1) Roadside ditch. 8.4 km SE of Harrell on St. Hwy. 160 (Sec. 4, T15S, R15W). 15 Jun. 1979. HWR. BYU (7). (Tumlison and Robison 2010).
(2) Roadside ditch, 10.1 km SE of Harrell on St. Hwy. 160. 23 Mar. 1986. HWR. SAU (1 ovigerous female).
(3) Locust Bayou at St. Hwy. 278, E of Camden. 8 May 1997. HWR. SAU (2).

Chicot County (*n* = 1)
(1) Unnamed creek, 9.7 km N of Lake Village (33.4132°N, 91.3184°W). No date. RR (Reimer 1963). (1).

Clay County (*n* = 1)
(1) St. Francis River at Greenway & Big Bay (36.3168°N, 90.0813°W). No date. (Meek 1894). (1).

Columbia County (*n* = 2)
(1) Roadside ditch, 1.1 km W of Magnolia on U.S. Hwy. 82 (Sec. 34, T16S, R21W). 26 Apr. 1982. (Tumlison and Robison 2010). HWR. BYU (2).

Crittenden County (*n* = 1)
(1) Unnamed creek, 17.9 km N of Marion (35.3646°N, 90.2544°W). No date. RR (Reimer 1963). (1).

Desha County (*n* = 1)
(1) Unnamed creek, 2.9 km E of Dumas (33.8817°N, 91.4513°W). No date. RR (Reimer 1963). (1).

Hempstead County (*n* = 2)
(1) Tributary to Bois d'Arc Creek at jct. of St. Hwys. 4 & 73 (33.6926°N, 93.6368°W). No date. RR (Reimer 1963). (1).
(2) Boat launch at Beard Lake (33.697°N, 93.943119°W). 30 Jun. 2017. HWR and C.T. McAllister. (1).

Jefferson County (*n* = 5)
(1) Unnamed creek, 7.6 km W of Pine Bluff on U.S. Hwy. 65 (34.3117°N, 92.1063°W). No date. RR (Reimer 1963). (5).

Lawrence County (*n* = 1)
(1) Unnamed creek, 3.2 km SE of Hoxie, off St. Hwy. 5 (36.0270°N, 90.9315°W). No date. RR (Reimer 1963). (1).

Lincoln County (*n* = 52)
(1) Bayou Bartholomew off St. Hwy. 54 at Garrett Bridge (33.8666N, 91.6562°W). 18 Aug. 1974. HWR. USNM 146064. (3, 1 male II, 2 females).
(2) Bayou Bartholomew at St. Hwy. 293, 1.6 km S of jct. of AR St. Hwys. 293 and 11 (33.9532°N, 91.7335°W). 18 Aug. 1974. HWR. USNM 146076, 146569. (39, 1 male I; 19 male II; 20 females).
(3) Long Lake at Woodville off St. Hwy. 11, E of Dumas. 7 Apr. 2014. HWR. SAU. (3 females).
(4) Silver Moon Lake beside St. Hwy. 212, E of Dumas. 29 Jun. 2014. HWR and CT McAllister. USNM 146569. SAU. (6, 2 Form II males and 4 mature females).

Mississippi County (*n* = 2)
(1) Pemiscot Bayou at U.S. Hwy. 61, *ca.* 3.2 km N of Blytheville. No date. HWR. SAU. (2 juvenile females).

Monroe County (*n* = 28)

(1) Flint Creek, 3.2 km E of Brinkley (34.9046°N, 91.1351°W). No date. RR (Reimer 1963).
(2) Roadside ditch, 14.6 km SE of Clarendon on St. Hwy. 17. 10 Jul. 1993. HWR. (34.6088°N, 91.1957°W). SAU (1 mature female).
(3) Roadside ditch, 7.1 km W of Clarendon on St. Hwy. 79. 15 Jul. 1994. HWR. SAU. (26, [4 Form II males, 7 mature females, 15 juveniles]).

Phillips County (*n* = 1)
(1) Big Creek at Poplar Grove (34.55544°N, 90.8458°W). 24 Jul. 1973. HWR. USNM 206037 (1 juvenile male)

Poinsett County (*n* = 2)
(1) Unnamed creek, 6.4 km W of Harrisburg (35.5649°N, 90.8264°W). No date. RR (Reimer 1963).
(2) St. Francis River. 17 Oct. 1987. GL Harp. USNM 219787 (1).

St. Francis County (*n* = 1)
(1) St. Francis River, *ca.* 183 m (200 yds.) N of I-40 bridge (35.0373°N, 90.7501°W). 28 Sept. 1974. HWR. USNM 146735. (1 male I).

Union County (*n* = 7)
(1) Flooded ditch, 2.4 km E of Strong on U.S. Hwy. 82 (Sec. 35, T18S, R12W). 10 Jul. 1993. HWR. BYU (3). (Tumlison and Robison, 2010).

A Description of Variation in Fecundity Between Two Populations of Wolf Spider *Rabidosa rabida* in Searcy Arkansas Using Brood Size Measurements

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Running title: Fecundity Variation in Local *R. rabida* Populations

Abstract

Fecundity, a very important population variable, can be estimated by measuring the number of juveniles hatching out of individual egg sacs. *Rabidosa rabida* is a large wolf spider that is common in Arkansas and much of the eastern portion of North America. This study attempts to expand previous estimates of variation in fecundity made for this species by Reed and Nicholas in Mississippi. In an attempt to determine baseline variation in a common arthropod predator, we hypothesized that a significant variation would be found in fecundity estimates between two populations of *R. rabida* in Arkansas. We also hypothesized that this variation would be similar to the variation reported in Mississippi. Two populations of *R. rabida* were collected in late August and early September of 2016. The egg sacs were allowed to hatch while both the mothers and juveniles were placed in alcohol, with the exception of twenty from each mother which were photographically measured. A comparison was made between the two populations and between variation measured by Reed and Nicholas. We found significant variation between brood size of the two populations in Arkansas similar in magnitude to what was found in Mississippi. We did not find any significant difference in size of juveniles or mothers between the two locations similar to what was found in Mississippi. Observing patterns in these traits provide a starting point for comparison to future measurements which may aid in quantifying differences in populations. A lack of descriptive data for arthropod species has been a challenge in ecological and conservation studies.

Introduction

Fecundity is defined as "the actual reproductive rate of an organism or population, measured by the number of gametes (eggs), seed set, or asexual propagules by an organism" (Van de Valle 1982). Since fecundity is highly plastic, meaning that it can be manipulated or affected by changes in the environment, it is widely studied in many different organisms. Fecundity studies help researchers gain insight into various life history traits as fecundity is directly related to the amount of energy involved and distributed within certain species (Llodra 2002; Head 1995). Fecundity of Arthropods has been a focus of biology for determining life history traits, including the role of diapause due to climate change (Llodra 2002; Head 1995). Insect and Arthropod life cycles necessitate a dormant phase which occurs during the winter (Bale and Hayward 2010). This period of diapause is important for the cycle of development of various organisms. Spider survivorship and propagation can be influenced by a variety of environmental conditions such as temperature, prey availability (Nyffeler and Birkhofer 2017), and home range from the nesting site. *Rabidosa rabida* is large wolf spider found across eastern North America. This spider is found in the grasslands and areas of low vegetation, (Brady and Mckinley 1994, Eason and Whitcomb 1965). The wolf spider typically has a single reproductive event during which the female produces a large number of offspring. A small number of offspring are predicted to survive until maturity, showing a type III survivorship curve (Edgar 1971). Brood size, or the number of juveniles produced in a single reproductive event, can be seen as a positive correlation with size of the mother. Brood size can also be correlated with environmental influences, such as temperature and elevation which affect overall metabolism, thus affecting energy allocation and overall reproduction (Punzo and Farmer 2006; Bale and Hayward 2010; Bonte *et al.* 2008). Reed and Nicholas (2008) researched gene flow and fecundity within two populations of *R. rabida* geographically located in Mississippi. In our study, we wanted to expand upon previous research by examining the same species for similar fecundity variation in different geographical areas within the described distribution of

R. rabida outside of Mississippi. Our secondary goal was to provide illustrations of the current changes in spider fecundity as well as highlight potential change that could occur in respect to future research on this topic. We hypothesized that there would be significant difference between mother size and brood size with no significant difference in offspring size and location similar to the work done in Mississippi. In our study, we measured fecundity as a means of predicting future applications of life history traits over multiple generations. Descriptive biological research of *R. rabida* is lacking. Further description and research is necessary to describe a better understanding of these fecundity changes during times of environmental change.

Materials and Methods

Spiders were collected from 2 locations in Searcy, Arkansas in White Co. using the spotlight method (Wallace 1937; Eason and Whitcomb 1965). Spider collection sites were chosen based on the proximity to the university lab and legal permissions available to collect samples. Ecologically these sites were similar to sites studied by Reed and Nicholas. The first location was along a bike path running through Berryhill park (35.260680, -91.718951) and the second location was a powerline right of way on land owned by Harding University on the west side of Searcy (35.354529, -91.641870). Beginning in late August, 47 spiders without egg sacs were collected and kept in the lab at Harding University in 16x14.5x8 cm clear plastic boxes with water provided ad libitum via shell vials stoppered with cotton. These spiders produced egg sacs while in captivity. As soon as juveniles emerged and the egg sac was dropped, the mother and offspring were placed in isopropyl alcohol and the date of egg sac creation, hatching, and preservation were recorded for later analysis. Photograph measurements of the juvenile spiders were taken utilizing a millimeter ruler underneath a clear petri dish in which the spider was placed (Figure 1).

In early September, we went back to Berryhill park and Gilliam farm and collected spiders that had already put out egg sacs and brought them to the lab. Juveniles emerged and were placed in isopropyl alcohol following the same methods as described above. When 47 spiders had been preserved, we counted the number of juvenile spiders in alcohol and added the number kept out of alcohol for measurements and recorded these numbers. Measurements of the juveniles from each mother were also taken from the photographs.

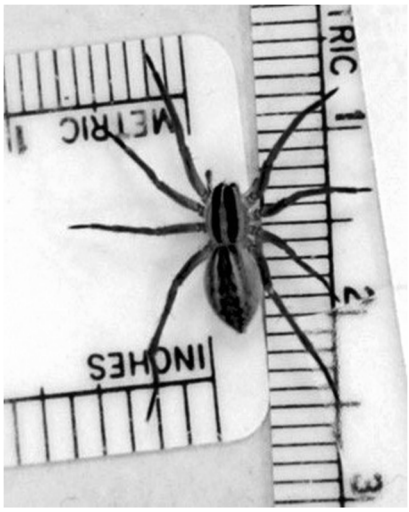

Figure 1. Photograph measurement of juvenile carapace length (millimeters).

We then used calipers to measure the carapace, length, width, and body length of each mother. We compared brood size between spiders who produced eggs sacs in the lab and in the field. We also compared carapace length of mothers from each sample location.

We performed an ANCOVA using brood size as the dependent variable, the location of spider capture as the independent variable, and the mother's carapace length as the covariate. Carapace length (CL) was used rather than the more traditional carapace width (CW) due to CL having a greater significant impact than CW (Stork 2011). A second ANCOVA was performed using the mean CW of the juvenile spiders from each mother as the dependent variable, the location of mother's collection as an independent variable, and mother's CL as a covariate. We performed an ANOVA using mother carapace length as the dependent variable and location as the independent variable. Descriptive statistics for brood size and juvenile size were calculated and graphed. SYSTAT software was used for all statistical analysis guided by James F. Rohlf (2001). Results were compared to data previously reported by Reed and Nicholas (2008).

Results and Discussion

We found significant difference in the brood size between locations (ANCOVA: $p=0.004$, $MS=66{,}805.366$, $N=47$). The mean brood size per egg sac for the Berryhill Park population was 330.391 ± 108.612 and 262.375 ± 77.366 for the Gilliam Property population (Figure 2).

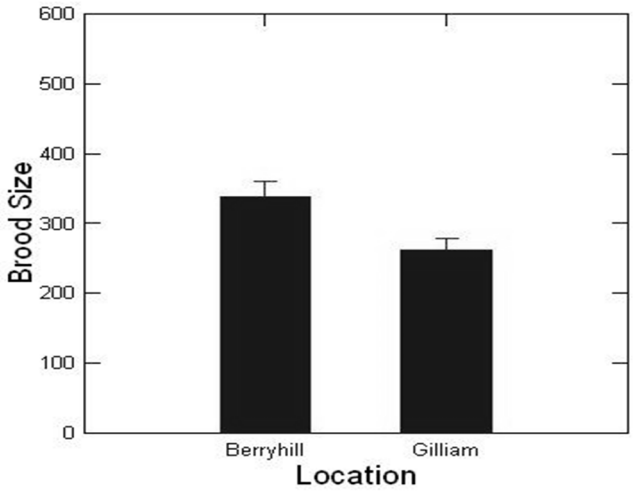

Figure 2. Differences in Brood Size based on location. Standard deviation bars are indicated.

We did not find significant difference in the mean body length of the juvenile spiders measured immediately upon exit from the egg sac between locations (p=0.306, MS=0.495, N=940). The mean body length of the juveniles in each egg sac was 5.113 ± 0.688 mm for the Berryhill population and 5.465 ± 0.636 mm for the Gilliam population (Figure 3).

We did not find a significant difference in the mean carapace length of the mothers between locations. (p=0.078, MS=1.541, N=47).

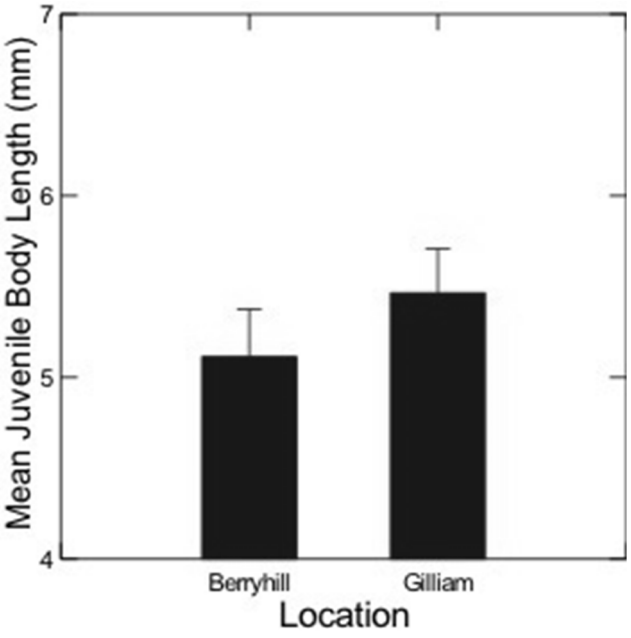

Figure 3. Mean body length of juveniles by location. Standard Error bars are indicated.

Table 1. ANOVA differentiating adult female body mass and brood size between two populations in Mississippi. Modified from Reed and Nicholas 2008.

Adult female mass	**d.f.**	**F-ratio**	**P**
Site	6	4.98	<0.0001
Year	2	7.73	<0.001
Site x Year	12	0.86	=0.59
Brood Size	**d.f.**	**F-ratio**	**P**
Site	6	4.40	<0.0005
Year	2	3.72	<0.03
Site x Year	12	0.65	=0.79

While Reed and Nicholas compared mass of female spiders and mass of juveniles we measured carapace length of females and body length of offspring, similar significant results were observed. Data from the Reed and Nicholas (2008) paper are included here for comparison (Table 1).

Discussion

Our results indicate variation in fecundity between two populations of *R. rabida* sampled in White County Arkansas. This led us to fail to reject our initial hypothesis that there would be significant variation between brood size in different geographic locations. We also failed to reject our hypothesis that female size would differ between locations. Reed and Nicholas used mass as the main variable in comparing female size. We believe that using the same variable, mass, we would have also obtained significant difference in female size. Increasing our sample size would also increase the power of our comparison. (N=47, p=0.07). *R. rabida* has shown a great deal of variation in physiological, behavioral, and life history traits (Reed and Nicholas 2008). This variation appears to be consistent with the variation in brood size that was seen by Reed and Nicholas in Mississippi.

Our results show that brood size variation is consistent at least within the south central United States. future research may identify different patterns of variation across the reported range of this species (Brady and McKinley 1994). We suspect that the large range of this species and the limited gene flow might suggest the presence of multiple species and thus indicate different life history and phenology patterns (Reed and Nicholas 2008).

In our work with *R. rabida*, we often collect hundreds of spiders. We were concerned that removing large numbers of individuals from these populations might influence population size. The large number of juveniles per egg sac suggests that collecting spiders for future tests is not likely to negatively impact the population size due to their ability to produce large numbers of offspring. Future fecundity and juvenile size studies are planned to continue in white county for the next several years. Research on food availability and environmental factors such as precipitation and temperature will also be conducted. In addition, feeding tests will also be conducted in order to see if variation exists that might not be due to food availability alone.

Variation in mother size and fecundity alone are not enough to determine if there is potential for evolution due to changing selective pressures. Reed *et al.* (2007) have suggested that heritability of fecundity traits can be seen through population size and prey availability in an ever-changing environment. Further studies need to be conducted in order to further determine how genetic heritability plays a role in fecundity distribution. Fecundity characteristics such as brood size in arachnid populations provide a glimpse into population dynamics that lead to questions concerning evolutionary strategies of arthropods as their environment changes over time.

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A First Look into the Microbial Community of *Rabidosa rabida*, a Wolf Spider in Searcy, Arkansas

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Running Title: Microbial Community of *R. rabida*

Abstract

Many diverse animal models have been used to explore the interactions between host organisms and their microbiota. Increased understanding of microbe-host interactions could lead to improved healthcare and drug development. Spiders have venom, digestive fluid, and body fluid components that have been suggested to possess antimicrobial properties that could lead to new and interesting host-microbe interactions. While studies have been published on interactions between bacteria affecting the immune function and behavior of spiders, the spider microbiome has not been established to date. Excreta and body swabs were collected from *Rabidosa rabida*, a wolf spider typically found on tall grass or low vegetation. Bacteria were cultured on tryptic soy agar, an all-purpose media known to grow most common bacterial strains, plates and 53 bacterial samples were Gram stained, catalase, and coagulase tested using aseptic technique. *Staphylococcus aureus*, *Staphylococcus* sp., and a Gram-positive bacillus were found on the excreta samples while *Staphylococcus sp*., Gram-negative bacilli, and Gram-negative cocci were found on the body swabs. Most of the excreta samples had little to no growth. The body swabs had multiple types of microorganisms that were limited to body location. A better understanding of this relatively simple host-microbe interaction can provide an understanding of the factors affecting these interactions allowing us to then understand more complex interactions such as those found in humans.

Introduction

In recent years, the symbiotic relationships between humans and microbes have become an area of focus for researchers (Li et al. 2008). With this growing interest on the microbiome, researchers have decided to focus on identifying members of the microbe community in hosts, to obtain insight into the ecological and evolutionary host-microbiota interactions in nature (Chow et al. 2010). The identification of microbial members in a host can lead to an understanding of the complex host-microbiota interactions which can eventually lead to personalized healthcare and to new targets for drug development for numerous systemic infections in humans (Kinross et al. 2011).

In this paper, microbiome is defined as the vast collection of aggregated symbiotic microorganisms harbored internally and externally by a host (Kinross et al. 2011). Numerous studies have suggested that the microbiome especially that found in the gut, has been the culprit for major health issues (DiBaise et al. 2008; Vrieze et al. 2010). Insect models in microbiome studies vary greatly in morphology and physio-chemical properties from humans, but can help researchers by providing answers to basic interactions between hosts and their microbial symbionts (Engel and Moran 2013).

Charroux and Royet (2012) studied Drosophila, a widely-used model for the study of developmental diseases, to determine advantages of gut microbiota. This led to the discovery that a very specific microorganism had a role in maintaining intestinal homeostasis. Researchers found that bumblebees' microbiota provided protection against the Trypanosome gut parasite *Crithidia bombi*. Koch and Schmid-Hempel (2011) also found that social contact between bees was necessary for the establishment of the protective microbiota in the gut. Researchers also found that bacterial communities in the gut of closely related species of the genus *Nasonia* assisted in the speciation and evolution of this genus (Flintoft 2013). Studies such as these and many more have given researchers a better understanding of these relationships (Potrikus and Breznak 1981; Dillon et al. 2000).

In addition to bees and wasps, spiders have also been studied to determine behavior and immune function as a result of infection with bacteria. (Gilbert et al. 2016; Keiser et al. 2016). However, little to no research has been conducted focusing solely on the microbiome of the spider. A few studies have been completed on spider venom and its components

including lycotoxins. (Yan and Adams 1998; Kuhn-Nentwig et al. 2002). Assays by Yan and Adams (1998) demonstrated some pore-forming activity against bacterial and yeast cell membranes that potentially makes these proteins from the venom of spiders antimicrobial in nature. Due to the antimicrobial potential of venom, researchers were curious to determine if spiders carry bacteria near their fangs.

Rabidosa rabida is a large wolf spider found across eastern North America that prefers tall grass and low vegetation (Brady and McKinley 1994). Spiders, like *R. rabida*, have a complete and relatively simple gut (Foelix 1996). *R. rabida*'s uses extra-oral digestion, where digestive fluid is expelled onto the prey and the liquefied contents are suctioned with a muscular pump called the sucking stomach initiating the catabolism of food. (Zibaee et al. 2012). Food remnants are then held in a pocket lined with cuticle before secretion occurs (Foelix 1996). In this study, we analyzed, for the first time, some of the microorganisms living on and in *R. rabida* with the use of standard microbiology methods. We hypothesized that there would be no microbial growth due to the antimicrobial properties of various spider body fluids.

Methods

Adult or nearly mature *R. rabida* were taken from tall grasses and low vegetation along the biking trail North of Berry Hill Park (35.261, -91.719) in Searcy, White County Arkansas after dark. The spiders were collected beginning in late June through early July of 2016. Maturation generally occurs in late July and August. The spotlight technique described by Wallace (1937) was used to locate and collect spiders. Captured spiders were immediately placed in sterilized collecting tubes and taken to the lab. In our first trial, excreta was collected using UV-sterilized plastic bags. Thirty spiders were placed in plastic bags where they were rearranged so that posterior end of abdomen faced an uncontaminated or sterile surface of the bag. Spiders were kept in that position until they excreted contents. Excreta was collected immediately to prevent contamination due to spider movement. Spiders (N=7) that did not excrete were excluded from this study. The plastic around the excreta sample was cut enough for the inoculation loop to reach the sample to prevent contamination.

In our second trial autoclaved microcentrifuge tubes were placed on the posterior end of the abdomen of 30 spiders until excretion occurred. Spiders were taped to a sterile surface. Microcentrifuge tubes were placed so that the excreta could be collected making sure that the spider's exoskeleton did not come in contact with the sample. The excreta were transferred onto tryptic soy agar (TSA) using a sterile inoculating loop via aseptic technique. Plates were incubated for 48 hours at 25ºC. The 25ºC incubator was used because in preliminary experiments fungi growth occurred at higher temperatures within hours, before analysis of bacteria could be performed. After this time, the plates were checked for growth and recorded. Each morphologically different colony was plated separately by streak plate method and incubated for another 48 hours. Gram stains, coagulase, and catalase tests were performed on pure cultures.

Sterile cotton swabs, moistened with sterile water were used to collect samples from the body surface of 3 spiders at five different locations. The body swab samples were transferred into tryptic soy broth (TSB) and incubated at 25ºC for 48 hours. Colonies were then transferred onto TSA plates and the broth was retained as stock culture. The pedipalps, prosoma, also known as the anterior body segment, abdomen, feet and rear, or posterior end of the abdomen around the anus and spinnerets were sampled (Figure 1). The body swab samples were kept in TSB incubated at 25ºC for 48 hours. Cultures were then transferred onto plates and the TSB was retained as a stock culture.

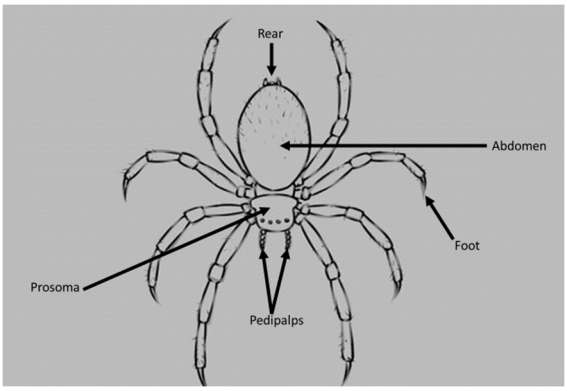

Figure 1. Location of Body swab samples from spider body (drawing adapted from lightofunity.us)

The fresh cultures from both the excreta and body swabs samples were stained with crystal violet to determine morphology and Gram stained to determine cell wall structure. Depending on their stain results, differential biochemical tests were performed. The Gram-positive cocci were tested for catalase and the analysis was recorded. The catalase positive cultures were then tested for coagulase. Bacteria were tested for

staphylocoagulase using a latex agglutination test specific for *S. aureus* surface proteins, a technique used in the further identification from a *Staphylococcus spp*. to *S. aureus* (Idelevich et al. 2014). The data was graphed according to the number of spiders showing each microbial type. Due to time constraints, the gram negative cocci bacteria were not further analyzed. Spiders that did not have bacterial colonies were also included. Data were graphed to show prevalence and variation of individual bacterial types within the spider population. In total 53 excreta samples were collected from both trials.

Results

Of the 53 total excreta samples, 40 showed no growth, 5 grew a single Gram-negative bacillus, 6 grew *Staphylococcus* sp. and 2 grew *Staphylococcus aureus* (Figure 2). Only one bacteria type was found from each sample.

Body swabs taken from 3 spider bodies made up 15 samples in total. The prosoma, abdomen and feet of each spider grew Gram-positive bacilli. Samples from the posterior end of the abdomen grew *Staphylococcus sp*. The samples from the pedipalps showed no bacterial growth. Fungal spores were found in all body swabs, but were not identified during this study.

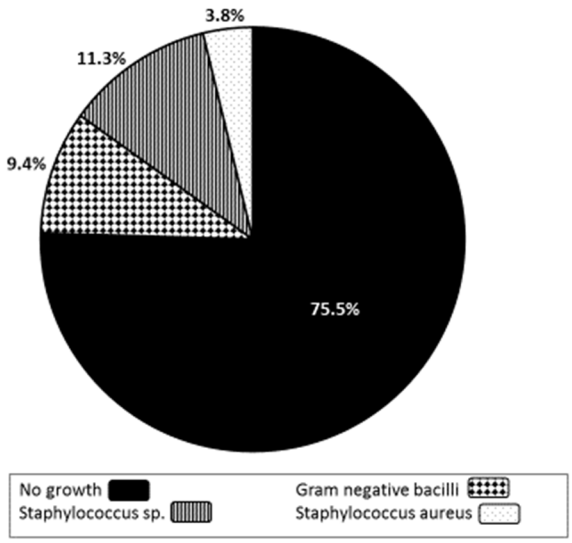

Figure 2. Comparison of the number of microorganism types that grew from the excreta samples from *R. rabida*

Discussion

Microbial growth was observed from the excreta of the spiders leading us to reject our hypothesis that there would be no microbial organisms in the excreta. Researchers were concerned with potential contamination of excreta samples collected from the inside of the bag the spiders were placed in. We attempted to collect only samples from excreta droplets located in uncontaminated areas. Spiders excrete forcefully and the excreta droplets could be collected further away from the spiders (Seitz 1987).

Excreta samples from both trials did not show significance so the samples were grouped together. The majority of the excreta samples did not grow any observable microbes and in those that had microbes present only a single type of bacterium was identified per sample. We hypothesize that the number of microbes present may be affected by the antimicrobial properties of venom (Budnik et al. 2004), digestive fluid and other body fluids.

In contrast to the excreta, the majority of the body swab samples, except the pedipalps, grew one or more organisms. The pedipalps, located in close proximity to the mouth, may be in contact with venom which is proposed to contain antimicrobial proteins (Yan and Adams 1998). In future tests, we plan to look at the effect of venom and other body fluids on the survival and growth of microbes.

Due to time constraints, we were not able to identify the majority of the microbes to species level. However, we did identify *Staphylococcus aureus*, which was found only in the excreta. *S. aureus* is a firmicute bacterium commonly found in the environment known to cause staph infection in humans (Foster 1996). More research is needed to see if this spider is a carrier for this potential pathogen.

S. aureus was only cultured from the excreta samples. The prosoma, abdomen and feet of each spider grew Gram-positive bacilli, while samples from the posterior end of the abdomen grew *Staphylococcus sp*. Pedipalp samples showed no bacterial growth. These differences show a spatial ecology that should be explored. This is not an exhaustive look of the microorganisms found in and on the spider. Different types of culture media with different pH levels, oxygen levels and nutrient content could be used to obtain a better understanding of the scope of the spider microbial community. In addition, researchers plan to obtain 16S rRNA sequencing to identify the bacteria living within and on the wolf spiders. Identifying the bacteria to species level will aid in determining if spiders are carriers of potential pathogenic bacteria as well as providing information related to spider habitat and movement patterns. With a better understanding of this relatively unknown spider-bacterial relationship, we can

better understand behavioral and social effects bacterial communities have on their host organisms. From symbiotic relationships to harmful parasitic relationships, bacteria may control more aspects of spider physiology and behavior, (Gilbert et al. 2016; Keiser et al. 2016), than is currently realized and thus allow us to broaden our understanding of more complex bacterial-host relationships.

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Bond Length and Bond Valence Relationships for Chromium Oxides, Chromium Sulfides, Molybdenum Oxides, and Molybdenum Sulfides

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Running Title: Bond Length and Bond Valence Relationships for Oxides and Sulfides of Chromium and Molybdenum

Abstract

Pauling determined an empirical logarithmic dependence of bond order (bond valence), *s*, to bond length, *R*, s = exp(R_0 – R/ b), where R_0 is unit bond length and *b* is a fitting parameter. Recently, an expression was derived for relating the *b* fitting parameter to theoretically derived atomic orbital exponents. With a method to calculate *b*, both R_0 and atomic orbital exponents can be experimentally determined through optimized fitting for Cr-O, Cr-S, Mo-O, and Mo-S. In the present study, bond length – valence relationships are found for Cr-O, Cr-S, Mo-O, and Mo-S chemical bonds using published crystallographic data. In addition, atomic orbital exponents were found for chromium and molybdenum: ζ_{Cr} = 1.247 and ζ_{Mo} = 1.381. Finally, bond lengths of unit bond valence, or true single bonds, were found using the bond valence model: R_o(Cr-O) = 1.770 Å, R_o(Cr-S) = 2.159 Å, R_o(Mo-O) = 1.893 Å, and R_o(Mo-S) = 2.264 Å.

Introduction

The oxides and sulfides of chromium and molybdenum are utilized as catalysts for many industrially important reactions such as the oxidation of methanol to formaldehyde (Ivanov *et al*, 1998; Klissurski *et al.*, 1993; Weisser and Landa, 1973). Consequently, the molecular structures, bond valences, and oxidation states of the catalytically active species is of keen importance. A method that has been very successful in relating bond lengths to bond valences (bond orders) and in determining oxidation states is the bond valence method (Brown 2002).

In 1947, Linus Pauling developed the following valence sum rule:

$$V_i = \sum_j s_{ij} \quad (1)$$

This relationship states that the total valence (*V*) of an atom will equal the sum of the individual bond valences (s_i) The rule parallels Kirchhoff's law which states the total current at a junction (ie., an atom) is the sum of the individual currents meeting at that point (i.e., the chemical bonds). The valence of different elemental systems can be related to bond lengths using Pauling's empirical relationship:

$$s = \exp\left(\frac{R_o - R}{b}\right) \quad (2)$$

where *s* is the bond valence, sometimes referred to as the bond order, R_0 is the length of a true single bond, R is the length of the individual bond associated with *s*, and *b* is the fitting parameter.

Recently, an equation was derived to calculate the *b* fitting parameter in Eq. (2) from an average of the atomic orbital exponents of the two bonding atoms (Hardcastle 2016):

$$b = \frac{a_o}{(\xi_{ave})} \quad (3)$$

The numerator a_0 is the Bohr radius of a hydrogen atom. The denominator is the average of orbital exponents for the two bonding atoms. Consequently, the bond valence *s* (or, bond order) between any two bonding atoms, given by Eq. (2), exponentially depends on the bond length *R* and the overlap of the electron density which is determined by the average of the orbital exponents (Hardcastle 2016). In this study, published crystallographically determined bond lengths from the oxides and sulfides of chromium and molybdenum are converted to bond valences using Eqs. (2) and (3), and normalized to the known valence (oxidation state) of the chromium or molybdenum atom using Eq. (1). Both, the R_o and Cr/Mo orbital exponent values are optimized to achieve a best-fit to the data.

Methodology

Data for bond lengths of the systems under study

were gathered from the Crystallography Open Database (Grazulis *et al.*, 2012). Crystallographic files (cif format) were utilized using the *Mercury 7* program. Bond lengths were recorded into an Excel spreadsheet, then corresponding bond valences were calculated using Eqs. (2) and (3). The bond valence sum was found using Eq. (1) and the error was determined by comparing to the expected (formal) oxidation state (or valence) of the chromium or molybdenum atom. Both the value of R_o (bond length of unit valence) and the atomic orbital exponent of the Cr or Mo were adjusted to minimize the overall fitting error. Crystallographic bond lengths were recorded to a maximum of 4.5 Å. Bonds outside this range contribute only a negligible amount to an atom's total valence.

All crystallographic data, calculated bond valences, and calculated Cr or Mo atomic valences are tabulated in a Supplemental File which can be found at http://scholarworks.uark.edu/jaas/.

Results and Discussion

A best fit of 45 compounds and approximately 900 data points (see the Supplemental file) yielded the following bond valence-length relationships for Cr-O, Cr-S, Mo-O, and Mo-S bonds, respectively:

$$s_{Cr-O} = \exp\left(\frac{1.770 - R}{0.3316}\right) \qquad (4)$$

$$s_{Cr-S} = \exp\left(\frac{2.159 - R}{0.3308}\right) \qquad (5)$$

$$s_{Mo-O} = \exp\left(\frac{1.891 - R}{0.3373}\right) \qquad (6)$$

$$s_{Mo-S} = \exp\left(\frac{2.264 - R}{0.3164}\right) \qquad (7)$$

Orbital exponents for oxygen and sulfur have already been determined in previous studies to be ζ_O = 1.959 and ζ_S = 1.962 (Hardcastle, 2016). In the present study, the atomic orbital exponents for chromium and molybdenum were found to be ζ_{Cr} = 1.247 and ζ_{Mo} = 1.381 using Eq. (3). The bond lengths having unit valence (true single bonds) were found to be R_o(Cr-O) = 1.770 Å, R_o(Cr-S) = 2.159 Å, R_o(Mo-O) = 1.893 Å, and R_o(Mo-S) = 2.264 Å.

Table 1 shows an example from the Supplemental file and also demonstrates how the bond valence method can be used. For the compound Li_2MoO_4 (Yip *et al.*, 2010), Mo-O bond lengths are tabulated in the first column. Mo-O bond valences (bond orders) are calculated from the bond lengths using Eq. (6), and these are tabulated in the second column. The individual bond valences are added, using Pauling's valences sum rule Eq. (1), to find the total molybdenum valence of 5.996 valence units (third column), expected to be consistent with the formal oxidation state of 6 for the molybdenum cation.

Table 1: Mo-O Bond Lengths, Calculated Bond Valences, and Calculated Mo^{6+} Atomic Valence in Li_2MoO_4 (Yip *et al.*, 2010)

Bond Length (R)	Bond Valence (s)	Calculated Mo^{6+} Valence
1.759	1.512	
1.766	1.479	
1.769	1.465	
1.770	1.460	
3.316	0.011	
3.700	0.003	
3.712	0.003	5.996

Conclusion

In the present study, Pauling's valence sum rule, Pauling's bond length-valence relationship, a recently derived expression relating a fitting parameter to atomic orbital exponents, and published crystallographic data were used to find bond length – valence relationships for Cr-O, Cr-S, Mo-O, and Mo-S chemical bonds. In addition, atomic orbital exponents were found for chromium and molybdenum: ζ_{Cr} = 1.247 and ζ_{Mo} = 1.381. Finally, bond lengths of unit bond valence, or perfect single bonds, were found using the bond valence model: R_o(Cr-O) = 1.770 Å, R_o(Cr-S) = 2.159 Å, R_o(Mo-O) = 1.893 Å, and R_o(Mo-S) = 2.264 Å.

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Bond Length and Bond Valence for Tungsten-Oxygen and Tungsten-Sulfur Bonds

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Running Title: Bond Length and Valence for Tungsten-Oxygen and Tungsten-Sulfur Bonds

Abstract

In 1947, Linus Pauling presented an "empirical" dependence of bond valence (*s*, also referred to as bond order) and bond length *R*: $s = \exp(\frac{Ro-R}{b})$, where R_0 is bond length of unit valence and "*b*" is a fitting parameter. Recently, an expression was derived for relating the *b* fitting parameter to theoretically derived atomic orbital exponents. With a method to calculate *b*, both R_0 and atomic orbital exponents can be experimentally determined through optimized fitting for W-O and W-S bonds. In the present study, bond length – valence relationships are found for W-O and W-S chemical bonds using published crystallographic data. The atomic orbital exponent for tungsten was found to be $\zeta_W = 1.534$. Unit valence (single bond) bond lengths were found to be R_o(W-O) = 1.901 Å and R_o(W-S) = 2.307 Å.

Introduction

The oxides and sulfides of tungsten are utilized as catalysts for many industrially important reactions such as green catalytic oxidation processes (Dai *et al.* 2016). Consequently, the molecular structures, bond valences, and oxidation states of the catalytically active species is of importance. A method that has been very successful in relating bond lengths to bond valences (bond orders) and in determining oxidation states is the bond valence method (Brown 2002).

Linus Pauling developed the five basic rules for chemical bonding in 1929 (Pauling 1929). His second rule is the principle of neutrality which states that the principle of local charge neutrality, where the negative charge of each anion is neutralized by the neighboring positive charges of the cations, and the cationic charges are neutralized by neighboring anions. In other words, the total valence of an atom is equal to the sum of the atom's individual bond valences. This is commonly known as the valence sum rule.

In 1947, Pauling published his empirical bond length-bond valence exponential relationship (Pauling 1947).

$$s = \exp\left(\frac{R_o - R}{b}\right) \quad (1)$$

where *s* is bond valence, R_0 is the bond length at unit valence, *R* is bond length associated with *s*, and *b* is a fitting parameter. The range of the values for the fitting parameter "*b*" is anywhere from 0.25 to 0.65 Å. This led to many inconsistencies, hindering chemists from comparing values. As a result, the value 0.37 Å was proposed as a universal constant for the "*b*" parameter (Brown and Altermatt 1985), thereby leaving equation (1) with only one fitting parameter, R_0.

In 2016, Hardcastle derived Pauling's empirical bond length-valence equation using quantum-mechanical considerations. This resulted in an expression for the "*b*" fitting parameter that incorporates the atomic orbital exponents of the bonding atoms (Hardcastle 2016). The "*b*" parameter is now defined as

$$\mathrm{b} = \frac{a_0}{\xi_{ave}} \quad (2)$$

where "*b*" is determined by the Bohr radius, a_0 (0.529 Å), and the average of the atomic orbital exponents of the atoms contributing to the bond. This definition allows the "*b*" parameter to be specific to each type of chemical bond.

In the present study, published crystallographically determined bond lengths for tungsten oxides and sulfides are converted to bond valences using Eqs. (1) and (2), and normalized to the known valence (oxidation state) of the tungsten atom using Pauling's valence sum rule. The R_o and orbital exponent values are numerically optimized to achieve a best-fit to the crystallographic bond distance data.

Methodology

Data for bond lengths of the systems under study were gathered from the Crystallography Open Database

(Grazulis *et al*. 2009). Crystallographic files (cif format) were utilized using the *Mercury 7* program. Bond lengths were recorded into an Excel spreadsheet, then corresponding bond valences were calculated using Eq. (1). Crystallographic bond lengths were recorded to a maximum of 4.5 Å. Bonds outside this range contribute only a negligible amount to an atom's total valence.

All crystallographic data, calculated W-O and W-S bond valences, and calculated tungsten atomic valences are tabulated in a Supplemental File which can be found at http://scholarworks.uark.edu/jaas/.

Results and Discussion

Crystallographic information files (cif files) were collected from the Crystallographic Open Database (Grazulis *et al*. 2009). Single-crystal X-ray diffraction data was collected for tungsten-sulfur (W-S) and tungsten-oxygen (W-O) compounds. Eq. (1) was used to convert published bond lengths to bond valences. Using Pauling's valence sum rule, the total valence of the tungsten was calculated. Then the tungsten atomic orbital exponent and R_0 values were adjusted to minimize the overall error in the spreadsheet.

X-ray diffraction data, was collected for W-O, and W-S bonds lengths. Each environment is represented in Table 1. Data analysis and error minimization led to two formulas one for W-O bonds:

$$s_{W-O} = \exp\left(\frac{1.901 - R}{0.3030}\right) \qquad (3)$$

$$s_{W-S} = \exp\left(\frac{2.307 - R}{0.3027}\right) \qquad (4)$$

The "*b*" values for the W-O and W-S bonds are significantly lower than the assumed universal constant value Brown and Altermatt at 0.37 Å (Brown and Altermatt 1985).

Orbital exponents for oxygen and sulfur have already been determined in previous studies to be ζ_O = 1.959 and ζ_S = 1.962 (Hardcastle 2016). In the present study, the atomic orbital exponent for tungsten was found to be ζ_W = 1.409 using Eq. (2). The bond lengths of unit valence (true single bonds) were found to be R_o(W-O) = 1.901 Å and R_o(W-S) = 2.307 Å.

Table I shows an example from the Supplemental file and also demonstrates how the bond valence method can be used. For the compound O_8W_2Zr (Auray 1995) W-O bond lengths are tabulated in the first column. W-O bond valences (bond orders) are calculated from the bond lengths using Eq. (3), and these are tabulated in the second column. The individual bond valences are added, using Pauling's valences sum rule, to find the total tungsten valence of 6.14 valence units (third column), consistent with the formal oxidation state of 6 for the tungsten cation.

Table 1. W-O Bond Lengths, Calculated Bond Valences, and Calculated W^{6+} Atomic Valence O_8W_2Zr (Auray 1995)

Bond Length (R)	Bond Valence (s)	Total Valence
4.352	0.000307	
1.785	1.4674222	
1.785	1.4674222	
1.785	1.4674222	
3.624	0.0033933	
4.397	0.0002646	
1.736	1.7250002	
4.26	0.0004159	
3.624	0.0033933	
4.397	0.0002646	
3.624	0.0033933	
4.144	0.0006099	
4.26	0.0004159	
4.352	0.000307	
4.397	0.0002646	
4.563	0.000153	
4.26	0.0004159	
4.144	0.0006099	
4.144	0.0006099	
4.352	0.000307	
4.563	0.000153	6.142545

Conclusion

Bond valence—length relationships provide a way to predict validity of proposed crystal structures when used with the valence sum rule. In this study, atomic orbital exponents were used to calculate "*b*" parameters for W-O and W-S bonds: 0.3030 Å and 0.3027 Å, respectively. This parameter was formerly treated as a universal constant. Bond valence—length relationships for W-O and W-S were determined by using published crystallographically determined bond lengths for W-S and W-O chemical bonds. The atomic orbital exponent for tungsten was found to be ζ_W = 1.534. Unit valence (single bond) bond lengths were found to be R_o(W-O) = 1.901 Å and R_o(W-S) = 2.307 Å.

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The Fishes of Chadron Creek, Dawes County, Nebraska

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Running Title: Fishes of Chadron Creek, Nebraska

Abstract

This first modern comprehensive survey of fishes collected from Chadron Creek, Dawes County, Nebraska, documents collections made with a small seine and backpack electrofisher during November 2007 and February and March 2008. Chadron Creek's fish community is of low diversity. The total of 3 collections at each of 9 stations along the length of Chadron Creek resulted in 254 individual fishes, which represented only 7 species within 4 families. Water quality parameters, including dissolved oxygen, pH, conductivity, total dissolved solids, temperature and fecal coliform counts indicate that Chadron Creek is a healthy stream capable of supporting a greater diversity of fishes. Land management practices may be responsible for elevated fecal coliform levels at one locality on the creek. Comparisons of fishes collected herein are made with historical records of fish collected between 1893 and 2000, and show that there are 50% fewer species present than those known from historical accounts.

Introduction

Fishes are important vertebrate components of any ecosystem's biodiversity. In order to maintain quality stewardship, management, and protection of these wildlife resources on public and private lands throughout the U.S., basic survey data are essential as a source of baseline information on species diversity, richness, and relative abundance. One such watershed is located in extreme northwestern Nebraska at Chadron Creek (Fig. 1), a small, perennial, spring-fed stream with headwaters near the top of the Pine Ridge escarpment about 19 km S of Chadron, Dawes County (Fig. 1). It continues north and northwest through Chadron State Park and converges with the White River a few km W of Chadron after it descends about 305 m (1,000 ft) in elevation. Chadron Creek is an important water resource for the region and is diverted for part of the municipal water supply and, to a lesser degree, for agriculture. The riparian areas along Chadron Creek contain chokecherry, cottonwood, green ash, hackberry, wild plum, and buffaloberry, and several introduced grass species. Sub-irrigated meadows lining its banks provide wildlife habitat and rangeland, two mainstays of the region's economy. Thus, as a field study area with relevance for scientific studies, Chadron

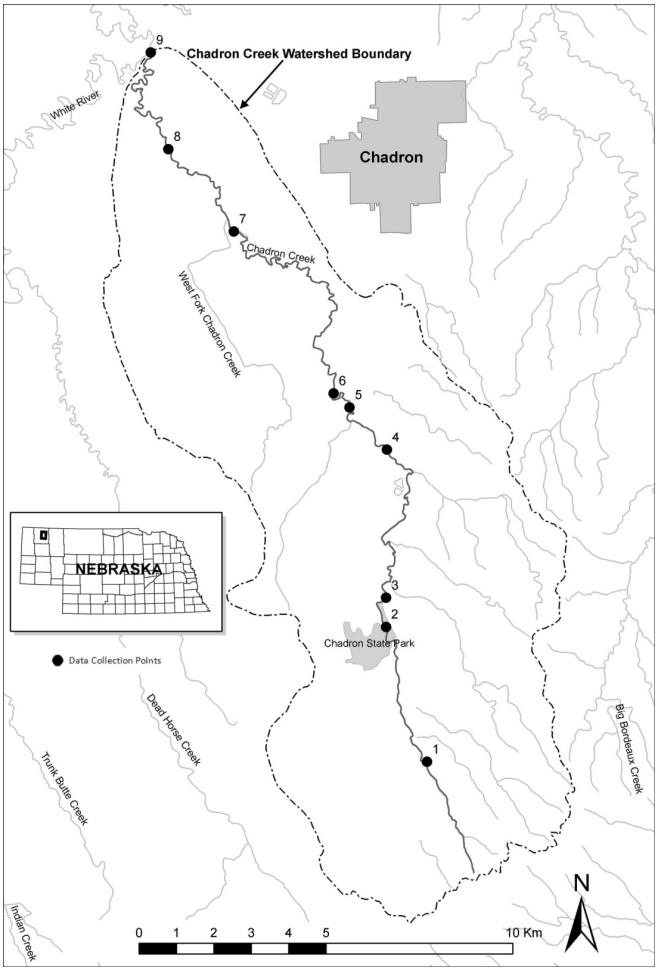

Figure 1. Nine localities in Chadron Creek, Dawes County, NE, where fishes were surveyed.

Creek is ideal.

To our knowledge, the first major report of fishes in the area was conducted by naturalists connected with the Pacific Railroad Survey parties, some of whom surveyed Chadron Creek in July 1893 and reported 9 species (Evermann and Cox 1896). Previously, P.H. Kirsch collected the Central Stoneroller (*Campostoma pullum*) in January 1893, and more than 4 decades later (July 1935), R. Van Dorp and P.M. Blossom collected Longnose Dace, *Rhinichthys cataractae* from Chadron Creek (S. Schainost, *pers. comm.*). Most interestingly, 3 specimens (USNM 76036 [2], UMMZ 245950 [1]) of Mountain Suckers (*Catostomus platyrhynchus*) were collected on 11 July 1893 and reported by Evermann and Cox (1896). The present occurrence of this species in Nebraska is considered extremely rare (Tomelleri and Eberle 1990) and it is listed as S1 (critically imperiled) in the state (Sowa *et al.* 2006). No mountain suckers have been collected in the state since 1939 when Raymond Johnson collected specimens from a tributary of Hat Creek, part of the Cheyenne River drainage (Schainost and Koneya 1999). Since it has not been collected in Nebraska for over 70 years, and since many sections of streams in which it was historically found now become seasonally dry, the species is considered extirpated from the state (Schainost and Koneya 1999; Belica and Nibbelink 2006). The most recent summary on fishes of the state was provided by Hrabik *et al.* (2012).

Since those initial surveys, aquatic habitats in the White/Hat Basin have been negatively impacted by agricultural pumping and demands for irrigation water. Early farmers built diversions and dug canals to farm the arid region. They reported that some of the flow of Chadron Creek was diverted during the irrigation season and that it flowed only during high runoff and some portions were dry the remainder of the year due to this irrigation. Indeed, for the first time in many decades, lower parts of Chadron Creek dried in the summer of 2007, just prior to this survey.

During various periods between 1973 and 2000, personnel from the Nebraska Department of Environmental Quality (NDEQ) and Nebraska Game and Parks Commission (NGPC) collected fishes from Chadron Creek (S. Schainost, *pers. comm.*). In addition, Brown Trout (*Salmo trutta*) were introduced into Chadron Creek in 1914 and Rainbow (*Oncorhynchus mykiss*) and Brook Trout (*Salvelinus fontinalis*) were collected in the 1990's (NDEQ samples). However, no modern comprehensive fish survey has been conducted and published in the refereed literature on Chadron Creek. Therefore, the purpose of our study was two-fold: (1) to provide a current baseline survey of the fishes of Chadron Creek and compare with previous unpublished reports, and (2) document information on water quality of the creek because it plays an important role in indicating the health of this watershed and its ecosystem.

Materials and Methods

Fifty meter segments (3 passes each) of Chadron Creek were surveyed at 9 sites along its length (when accessible) on 11 November 2007, 24 February 2008, and 30 March 2008 (see Fig. 1) at latitudes and longitudes as follows: **(1)** 42°40'55.31"N, 103°00'4.05"W; **(2)** 42°42'34.18"N, 103°00'34.56"W; **(3)** 42°42'37.52"N, 103°00'32.31"W; **(4)** 42°42'57.24"N, 103°00'43.12"W; **(5)** 42°43'6.38"N, 103°00'33.59"W, **(6)** 42°43'18.89"N, 103°00'34.236"W; **(7)** 42°49'15.20"N, 103°03'50.56"W; **(8)** 42°49'42.14"N, 103°04'38.00"W; and **(9)** 42°50'47.80"N, 103°04'42.06"W. These locations were logged using GPS, and characterized according to size, presence of fish/water, and types of aquatic vegetation.

The protocol for fishes involved careful, standardized field collections, species level identification, enumeration, and analyses using aggregated biological attributes or quantification of the numbers of key species (Barbour *et al.* 1999). Taxonomic keys were used to identify fish to species level and those measuring <20 mm were not included in analyses because of difficulty in verifying identification (particularly cyprinids). Dissection of pharyngeal teeth was necessary to verify the identity of some minnows. We used a backpack electrofisher (pulsed direct current) to obtain a representative sample of the fish assemblage at each reach that was isolated with block nets. Small nylon seines (3.1 m long × 1.8 m deep) with a 1.6 mm Ace mesh and dip nets were used when applicable. Attempts were made to collect fishes from each of the 9 sites and those collected were counted, preliminarily identified, sorted, and fixed in 10% formalin. They were transferred to 45% ethanol for storage prior to final identification in the laboratory. Voucher specimens were deposited in the Henderson State University (HSU) fish collection, Arkadelphia, Arkansas.

We analyzed water quality using standard chemical parameters (Standard Methods 2005) including dissolved oxygen, pH, conductivity, total dissolved solids, and temperature. Measurements represent an average of 3 readings from the lower, middle, and upper portions of each individual 50 m site, when accessible. In addition, counts of fecal coliform colonies were made

at sites 1–7 in Fall 2007 and Spring 2008 (site 7 was not sampled in Winter 2008 and samples were not obtained from sites 8 and 9 during any sampling period).

Families are arranged in phylogenetic order and species and common names are listed as given by Page *et al*. (2013). Fishes were collected by the senior author.

Results and Discussion

Aquatic Vegetation

Aquatic (floating and submerged) vegetation included duckweed (*Lemna turionifera*), submerged waterweed (*Elodea canadensis*), white water crow's foot (*Ranunculus longirostris*), emergent watercress (*Nasturtium officinale*), and cutleaf water parsnip (*Berula erecta*). This vegetation provided adequate cover for fishes to hide and feed on macroinvertebrates.

Fishes

The total of 3 collections at each of 8 of 9 stations along the length of Chadron Creek resulted in 254 individual fishes, which represented only 7 species within 4 families as follows.

CYPRINIDAE

***Notropis stramineus* (Cope, 1865) – Sand Shiner.** This shiner generally inhabits streams ranging from small spring discharges to large rivers where it usually is associated with sandy substrate in areas with little or no aquatic vegetation and moderate to slow current; however, it is rarely found in upland areas. It was originally reported from Chadron Creek by Evermann and Cox (1896) as *Notropis blennius* (Table 2). A single specimen was also collected in 1994 by Peters (REMAP, FHSM 3030) (Table 2). Although we did not collect specimens from the lower 4 sites on Chadron Creek, it appears to be a common shiner of the northern regions of this watershed similar to that described previously (Table 1). It is considered one of the most abundant fishes of medium-sized, sandy-bottomed rivers that are typical of the Nebraska prairie (Hrabik *et al*. 2012).

***Pimephales promelas* Rafinesque, 1820 – Fathead Minnow.** This is another cyprinid historically known from Chadron Creek (Table 2). Additional specimens were collected by NDEQ in 1993 (Table 2). However, as we collected only a single specimen during this study (Table 1), we suggest the species may be an uncommon inhabitant of this watershed. It is widespread in North America, inhabiting a wide variety of aquatic habitats, and tolerant of high temperatures, turbidity, and low oxygen. Fathead Minnows are considered to be common throughout Nebraska and are important as bait (Hrabik *et al*. 2012).

***Rhinichthys cataractae* (Valenciennes *in* Cuvier and Valenciennes, 1842) – (Longnose Dace).** Longnose dace have historically been reported from Chadron Creek since 1893 (Table 2). During our study, several specimens were collected from 5 of 8 (63%) sites in the watershed (Table 1). In the Horse Creek drainage of nearby eastern Wyoming, *R. cataractae* biomass was primarily related to submerged aquatic vegetation, main channel run habitat, and overhead cover features (Hubert and Rahel 1989). Very similar ecological conditions were present in Chadron Creek. Interestingly, *R. cataractae* has the widest range of any North American minnow (Page and Burr 2011) and it is common in streams of northwestern Nebraska (Hrabik *et al*. 2012).

***Semotilus atromaculatus* (Mitchill, 1818) – (Creek Chub).** The Creek Chub was not reported from Chadron Creek until 1993–1994 (NDEQ and REMAP samples, Table 2). We commonly found various age and size classes of this cyprinid (Table 1); it appears to be the most common species of this watershed. The Creek Chub often inhabits headwater creeks where there are few other fishes, a scenario found in Chadron Creek. It is common in many streams throughout Nebraska (Hrabik *et al*. 2012).

CATOSTOMIDAE

***Catostomus commersonii* (Lacépède, 1803) – (White Sucker).** The White Sucker has been known from Chadron Creek since 1893 (Table 2). There is an additional 1935 record collected by Van Dorp and Blossom from Chadron Creek in the UMMZ (UMMZ 108528). Two additional White Suckers were collected in 1993 and 2000 (Table 2). This sucker appears to be uncommon in Chadron Creek as only a single specimen was collected during this study (Table 1). Populations are apparently secure (S4) in the state (NatureServe 2015) and it is common in all drainages in Nebraska (Hrabik *et al*. 2012).

SALMONIDAE

***Salmo trutta* (Linnaeus, 1758) – (Brown Trout).** This fish is native to Europe, North Africa, and western Asia; it was introduced to North America around 1883

and now widely stocked throughout south Canada and much of the United States (Page and Burr 2011). Although Brown Trout was first stocked in Chadron Creek in 1914 (Hrabik *et al*. 2012), it was not until 1987 that they were first vouchered from the creek (Table 2) and appears to be perfectly suited for continual stocking into this cool, high gradient stream. Juvenile specimens of *S. trutta* were commonly collected in pools and riffles of 2 upper reaches of Chadron Creek in the vicinity of the State Park (Table 1) although the creek has 21 km of trout-supporting water. In addition, Brown Trout have been reported to have a negative effect on the nongame fish community via piscivory (Garman and Nielson 1982); however, we do not know what effect they might have on similar communities in Chadron Creek. We did not save voucher specimens of *S. trutta* (all were released) due to them being a game fish and to avoid negatively influencing the fisheries of the site.

GASTROSTEIIDAE

***Culaea inconstans* (Kirtland, 1840) – (Brook Stickleback).** The Brook Stickleback was not known from Chadron Creek until McAllister *et al*. (2010) documented a single specimen from this watershed. The species is considered S3 (vulnerable) in the state (NatureServe 2015) and is also listed as a Tier II, Species at Risk, in the state by the Nebraska Natural Legacy Project (Hrabik *et al*. 2012).

Conclusions

When comparing historical records of fishes from Chadron Creek (Table 2) to those reported in the present survey (Table 1), there are 50% fewer species present than were known from historical accounts. Of these, there are 3 more cyprinids, one catostomid, 2 (non-native) salmonids, one ictalurid, and one centrarchid known from historical records (Table 2). Although the Stonecat (*Noturus flavus*), *C. pullum* (as *C. anomalum*), Blacknose Shiner (*Notropis heterolepis* as *N. cayuga*), Flathead Chub (*Platygobio gracilis*), and *C. platyrhynchus* were reported from the original historical 1893 collections of Evermann and Cox (1896), they have not been collected since (over 100 yrs) in Chadron Creek proper. Unfortunately, voucher specimens of these collections have apparently been lost or discarded. Of these fishes, *N. heterolepis* and *C. platyrhynchus* are critically imperiled (S1) species in Nebraska (NatureServe 2015). In Missouri, the former species has apparently disappeared from several Ozark streams that were occupied prior to 1900 (Pflieger 1997). One additional fish, the Green Sunfish (*Lepomis cyanellus*) has also not been collected in Chadron Creek proper since the original collection of 6 specimens by NDEQ personnel in 1993. However, there is a 1935 collection of 4 *L. cyanellus* from the Chadron Creek watershed at McDowell's Pond, 8.0 km S of Chadron, and another collected in 1939 by R.E. Johnson and R. Wallace from the White River system of Missouri River drive (near the site of the current Chadron Reservoir and Chadron Reservoir #2). In addition, there is also a record of *R. cataractae* from the same latter site (UMMZ 134485).

There are also records of other fishes from near the mouth of Chadron Creek in the mainstem White River. The Red Shiner (*Cyprinella lutrensis*) and *N. flavus* were collected there after our survey period in July 2012 and are deposited in the Auburn University Museum of Natural History (AUMNH), Auburn, AL. Therefore, it is likely *C. lutrensis* will soon become a member of the Chadron Creek ichthyofauna and the Stonecat exists in a local extant source population.

The only 2 sites on Chadron Creek that did not support fish were sites 1 (Chadron Creek WMA) and 8 (US 80 bridge). The former location was a headwater site with flowing water and a small pond while the latter location was completely dry during sampling.

Water quality data (Table 3) suggest that Chadron Creek is a healthy stream capable of supporting a greater variety of fishes. In addition, microbiological analysis of fecal coliform colonies (Table 3) reveal very low values for sites 1–6; however, site 7 (Fall sample only) had very high values and suggests a difference in land use management with runoff from grazing livestock in the area. These sites with low fecal coliform counts were mostly drained by higher elevations in the National Forest or the State Park and, unfortunately, data is missing for site 7 in winter. However, site 7 did support a good population of *N. stramineus*, *R. cataractae* and *S. atromaculatus*.

We have provided the first definitive modern survey of the fishes of Chadron Creek. However, we freely admit that collection of several species (particularly minnows) may have been missed due to seasonal migration and/or use of differing winter habitats. Additional surveys should be conducted in the region (during all parts of the year, if possible) as some of the streams of the northwestern White and Hat river basins are remote and difficult to access. This could help determine whether or not *C. platyrhynchus* is actually extirpated in the state.

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Table 1. Fishes collected during this survey from Chadron Creek (2007–2008).

Species	Date collected	No. collected	Site(s)	HSU Cat. No.
CYPRINIDAE				
Notropis stramineus	Nov. 2007, Mar. 2008	40	5–6, 7, 9	3380, 3382
Pimephales promelas	Nov. 2007	1	5	3377
Rhinichthys cataractae	Nov. 2007, Feb.–Mar. 2008	33	2–4, 6–7	3378
Semotilus atromaculatus	Nov. 2007, Feb.–Mar. 2008	172	4–7	3379
CATOSTOMIDAE				
Catostomus commersonii	Nov. 2007	1	6	3381
SALMONIDAE				
Salmo trutta[1]	Nov. 2007, Feb.–Mar. 2008	6	2–3	–
GASTEROSTEIDAE				
Culaea inconstans[2]	Mar. 2008	1	5	3186

[1]Released.
[2]Previously reported by McAllister *et al*. (2010).

Table 2. Historical records of fishes collected from Chadron Creek (1893-2000).

Species	Date collected	No. collected	Cat. No. (if known)
CYPRINIDAE			
Campostoma pullum	1 Jan. 1893	4	USNM 76148[1]
	11 Jul. 1893	1	USNM 76198
Notropis heterolepis	11 Jul. 1893	1	–
N. stramineus	11 Jul. 1893	1	–
	12 Jul. 1994	1	FHSM 3030[2]
Pimephales promelas	11 Jul. 1893	1	–
	11 Aug. 1993	5	UNSM 6446[3]
Platygobio gracilus	11 Jul. 1893	1	MCZ 31722[4]
Rhinichthys cataractae	11 Jul. 1893	1	–
	5 Jul. 1935	10	UMMZ 108527[5]
	5 Jul. 1935	294	UMMZ 108529
	13 Aug. 1973	4	–
	5 Aug. 1992	4	UNSM 6179
	11 Aug. 1993	26	UNSM 6445
	12 Jul. 1994	658	UNSM 8708
	31 Oct. 2000	8	–
Semotilus atromaculatus	11 Aug. 1993	14	–
CATOSTOMIDAE			
Catostomus commersonii	11 Jul. 1893	1	CAS/SU 75147[6]
	5 Jul. 1935	1	UMMZ 108528
	11 Aug. 1993	1	–
	31 Oct. 2000	1	–
C. platyrhynchus	11 Jul. 1893	2	USNM 76036
SALMONIDAE			
Onchorhychus mykiss	12 Jul. 1994	1	–

Table 2 Historical records of fishes collected from Chadron Creek (1893-2000). *(cont'd)*

Species	Date collected	No. collected	Cat. No. (if known)
Salmo trutta	28 Jul. 1987	47	UNSM 4867
	12 Jun. 1990	26	–
	5 Aug. 1992	26	UNSM 6178
	11 Aug. 1992	26	–
	12 July 1994	29	UNSM 8709
	31 Oct. 2000	39	–
Salvelinus fontinalis	31 Oct. 2000	1	–
ICTALURIDAE			
Noturus flavus	11 Jul. 1893	1	–
CENTRARCHIDAE			
Lepomis cyanellus	11 Aug. 1993	6	UNSM 6444

[1]USNM = Smithsonian National Museum of Natural History, Washington, DC.
[2]FHSM = Fort Hays Sternberg Museum of Natural History, Hays, KS.
[3]UNSM = University of Nebraska State Museum, Lincoln, NE.
[4]MCZ = Museum of Comparative Zoology, Harvard University, Cambridge, MA.
[5]UMMZ = University of Michigan Museum of Zoology, Ann Arbor, MI.
[6]CAS/SU = California Academy of Sciences, San Francisco, CA.

Table 3. Selected water quality measurements taken in Chadron Creek (Fall 2007-Winter 2008).

Site No.[1]	1	2	3	4	5	6	7[2]
Fall 2007							
Water temp (°C)	11.8	9.7	9.9	11.7	9.7	10.5	7.8
pH	7.3	7.6	8.0	8.0	7.7	7.8	7.8
TDS (ppm)	194.7	226.3	222.5	233.0	245.0	254.5	271.8
Conductivity (µS)	386.1	456.8	444.6	476.2	493.8	508.9	538.4
Avg. fecal coliforms[3]	0.3	4.1	25.8	9.2	2.9	21.4	656.2
Winter 2008							
Water temp (°C)	10.1	5.1	5.3	6.5	5.8	5.7	–
DO (mg/l)[4]	11.5	11.4	11.3	11.9	12.3	13.0	–
pH	7.0	7.5	7.7	8.1	8.2	8.1	–
TDS (ppm)	193.3	227.0	227.7	233.9	232.4	237.2	–
Conductivity (µS)	400.6	444.0	445.2	463.8	459.5	482.9	–

[1]Sites 8 (dry) and 9 were not sampled for water quality. [3]Average fecal coliforms were not taken in winter sampling.
[2]Site 7 was frozen for winter sampling. [4]Dissolved oxygen (DO) measurements were not taken in fall sampling.

The Fleas (Arthropoda: Insecta: Siphonaptera) of Arkansas

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Running Title: Fleas of Arkansas

Abstract

Fleas (Insecta: Siphonaptera) are important ectoparasites of cats, dogs, other mammals (including humans), and birds, and are an important component of the biota of North America. In addition, they can be nuisance biters and serve as vectors or intermediate hosts of several flea-borne disease agents and parasites that negatively affect mammals and birds. In Arkansas, there have been no recent comprehensive summaries of fleas in the last 45+ years. Here, we provide a summary of the 29 species of fleas within 7 families that have been recorded from the state, update their taxonomy, and note their medical and veterinary importance.

Introduction

Fleas are small, wingless, hematophagous (blood-feeding) ectoparasites that mostly infest mammals (about 94% of known species), with the remainder of species parasitizing birds (Durden and Hinkle 2009). There are ca. 246 recognized genera and over 2,500 species within 16 families (Lewis 1998). Some species are notable nuisance biters of humans and domestic animals and some serve as vectors or intermediate hosts of flea-borne disease agents and parasites. Schiefer and Lancaster (1970) provided a checklist of the 21 species of fleas known at that time from Arkansas. However, their collections (made in 1968) were limited to sites in northwestern Arkansas. Since that paper, and despite the medical and veterinary importance of fleas in the state, there have been no attempts to provide a comprehensive list of Arkansas fleas.

The purpose of this report is three-fold: (1) produce an update on the fleas known to occur in Arkansas, (2) provide the most recent taxonomy on these fleas, and (3) note any known medical and veterinary importance of these fleas including species that are vectors for pathogenic microorganisms or intermediate hosts of parasitic agents.

Methods

We conducted an exhaustive search of the scientific literature and the world-wide web for information on fleas in the state. Records of recent collections of Arkansas fleas reported by us (McAllister *et al*. 2013; Connior *et al*. 2014; Tumlison *et al*. 2015) are also included. In addition, rodent trapping was conducted in Benton, Carroll and Saline counties during 2016 using Museum Special® snap traps and/or Sherman live traps baited with rolled oats.

Flea classification follows Lewis (1998). Common names of fleas listed herein follow the Common Names of Insects Database (Entomological Society of America 2016). Voucher specimens deposited in collections are designated (with accession numbers) as follows:
GSUENT: Georgia Southern University Entomology Collection, Statesboro, GA.
NMNH: Smithsonian National Museum of Natural History, Washington, DC.

Results

We report a total of 29 species of fleas within 7 families from Arkansas as follows (M = Male[s], F = Female[s]); coll: B.C. Marshall (BCM). Counties with records are provided in Fig. 1; most are in the Ozark Mountains physiographic region. However, not all localities are available from previous publications, including those for 4 species of fleas, particularly from south Arkansas in Pratt and Good (1954).

FAMILY PULICIDAE

***Cediopsylla simplex* (Baker) – rabbit flea.**

1M, 3F ex "rabbit," Lawrence Co., Imboden, Mar. 1925, coll: BCM (Fox 1940).

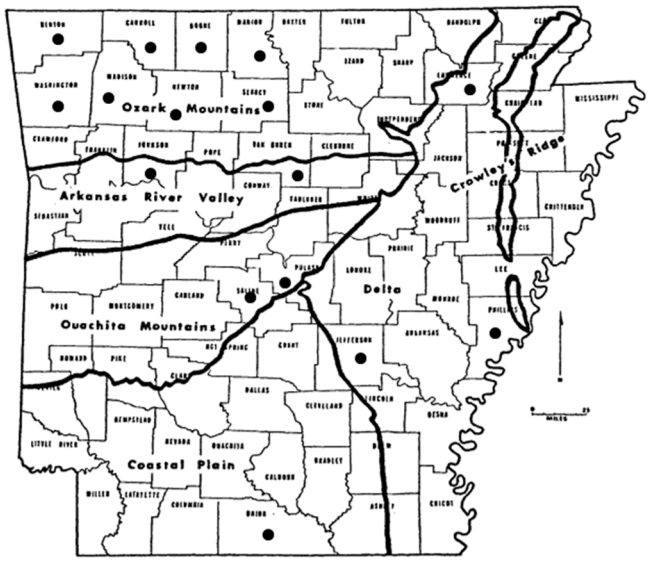

Figure 1. Arkansas counties within physiographic regions with flea records reported herein. Also see Discussion.

1F ex eastern cottontail (*Sylvilagus floridanus*, "Arkansas," coll: J.C. Bequeart (Hopkins and Rothschild 1953).

12M, 14F ex 7 *S. floridanus*, 3M, 8F ex 4 black-tailed jack rabbits (*Lepus californicus*), 2M, 8F ex 2 gray foxes (*Urocyon cinereoargenteus*), unreported numbers ex swamp rabbit (*Sylvilagus aquaticus*), 1968, NW Arkansas (Schiefer and Lancaster 1970).

Ex *S. floridanus*, "Arkansas" (Andrews *et al*. 1980).

This is a widespread flea of lagomorphs and their predators in the eastern two-thirds of North America (Holland 1985, Durden *et al*. 2012). Lagomorphs are reservoir hosts for *Francisella tularensis*, the causative agent of tularemia, and Hopla (1980a) provides evidence for the transmission of this bacterium by fleas.

***Ctenocephalides canis* (Curtis) – dog flea.**

Madison Co., Georgetown (no other data) (Trembley and Bishopp 1940).

1F ex unknown host, Lawrence Co., Imboden, 1928, coll: BCM (Fox 1940).

"Several specimens" ex domestic dog (*Canis lupus familiaris*), NW Arkansas, 1968 (Schiefer and Lancaster 1970).

The dog flea is a nuisance biter of domestic dogs and other canids. It is almost cosmopolitan in distribution and can serve as an intermediate host of both the double-pored dog tapeworm, *Dipylidium caninum* and dwarf tapeworm *Hymenolepis* (=*Vampirolepis*) *nana*. The latter, *H. nana*, is one of the most common cestodes of humans, especially young children in the southern U.S. A filiarial worm of dogs, *Dipetalonema reconditum*, is also transmitted by this flea (Durden and Hinkle 2009).

***Ctenocephalides felis* (Bouché) – cat flea.**

2F ex 10 striped skunk (*Mephitis mephitis*)*;* 15M, 68F ex 20 Virginia opossum (*Didelphis virginiana*)*;* 1F ex 17 brown rat (*Rattus norvegicus*); 1M, 1F ex 1 eastern spotted skunk (*Spilogale putorius*)*;* "numerous specimens" ex *C. l. familiaris;* 1M, 24F ex 15 domestic cats (*Felis catus*)*;* unreported numbers ex gray fox (*Urocyon cinereoargenteus*) and whitetail deer (*Odocoileus virginiana*); 1968, NW Arkansas (Schiefer and Lancaster 1970).

Benton, Johnson, Newton, Phillips, Saline, and Washington Cos., no host data (McKern *et al*. 2008).

Like the previous species, this flea is almost cosmopolitan. It is a vector of *Bartonella henselae*, the causative agent of cat scratch disease and of *Rickettsia felis*, and the agent of cat flea rickettsiosis (sometimes referred to as cat flea typhus) (Durden *et al*. 2012). Like *C. canis*, it can also serve as an intermediate host of *D. caninum* and *H. nana*, and can also transmit *D. reconditum.* This flea is a nuisance biter of domestic cats and wild felids and is also common on domestic dogs (Durden and Hinkle 2009).

***Echidnophaga gallinacea* (Westwood) – stick-tight flea.**

4F ex "dogs," Lawrence Co., Imboden, 27 Nov. 1931, coll: BCM (Fox 1940).

Lawrence Co., Imboden (no other data) (Trembley and Bishopp 1940).

Ex *R. norvegicus* and black rat (*Rattus rattus*), 5 localities across Arkansas (Pratt and Good 1954).

Ex *F. catus* and *C. l. familiaris*, "Arkansas" (Becklund 1964).

Ex *F. catus*, NW Arkansas, 1968 (Schiefer and Lancaster 1970).

This small flea is globally widespread in tropical, subtropical and warm temperate regions. It infests a wide diversity of avian and mammalian hosts (Durden *et al*. 2012). This flea has elongate, barbed mouthparts that allow it to embed in host tissue for prolonged periods, as reflected by its common name. It sometimes occurs in clusters of specimens on the host head where oral self-grooming cannot be directed against it by the host.

***Euhoplopsyllus glacialis affinis* (Baker) – no common name (NCN)**.

1M, 1F ex 20 *D. virginiana*; 2M, 2F ex 4 *L. californicus*; 18M, 25F ex 7 *S. floridanus*; 1M, 1F ex 2

U. cinereoargenteus; 1M, 1F ex 15 *F. catus*), NW Arkansas, 1968 (Schiefer and Lancaster 1970).

This flea is mainly associated with lagomorphs and mostly known from some mid-western states extending south to Texas (Hopkins and Rothschild 1953; Holland 1985). It has no known medical-veterinary importance although it could be an enzootic vector of *F. tularensis* (Hopla 1980a).

***Pulex irritans* Linnaeus – human flea.**

2F ex unknown host, Lawrence Co., Imboden, 1928, coll: BCM (Fox 1940).

Boone (no other data) and Madison Cos., Georgetown (no other data) (Trembley and Bishopp 1940).

9M, 11F ex 10 *M. mephitis*; 4F ex 20 *D. virginiana;* 1M, 1E ex 1 *S. putorius*; 1M ex 7 *S. floridanus;* 14M, 21 F ex 2 *U. cinereoargenteus;* "numerous specimens" ex *C. l. familiaris*; unreported numbers ex domestic cattle (*Bos taurus*) and bobcat (*Lynx rufus*), NW Arkansas, 1968 (Schiefer and Lancaster 1970).

The human flea is globally and widely-distributed mainly as an ectoparasite of medium-sized and large mammals (Hopla 1980b). It can serve as intermediate host of *D. caninum* and *H. nana*, which can also be transmitted to humans, especially children who have close contact with flea-infested cats and dogs. The role of this flea in human-to-human transfer of the plague bacterium is uncertain, but it is thought to be significant in some outbreaks (Hopla 1980b).

***Pulex simulans* Baker – NCN.**

2M, 6F ex 3 northern raccoons (*Procyon lotor*), Van Buren Co., 1989–1990 (Richardson *et al*. 1994).

This flea is widely distributed in the Americas as an ectoparasite of carnivores and some other medium-sized and large mammals (Hopla 1980b). Morphologically, it is very similar to *P. irritans* and prior to 1958, it was not recognized as part of the North American flea fauna. Other than biting domestic dogs and cats and sometimes humans (Durden *et al*. 2012), it has no known medical-veterinary importance.

***Pulex* sp.**

Ex *S. floridanus*, "Arkansas" (Andrews *et al*. 1980).

Females of *P. irritans* and *P. simulans* cannot be separated morphologically and prior to the paper by Smit (1958), all *Pulex* spp. fleas in North America were assigned to *P. irritans*. Therefore, any records of *P. irritans* prior to 1958 could actually represent either *P. irritans* or *P. simulans* and only collections that include male specimens can be identified with certainty since 1958. Therefore, some of the specimens recorded above as *P. irritans,* could have actually been *P. simulans.*

***Xenopsylla cheopis* – Oriental rat flea.**

Ex *R. norvegicus* and *R. rattus,* 23 localities across Arkansas (Pratt and Good 1954).

1M, 3F ex 17 *R. norvegicus*, NW Arkansas, 1968 (Schiefer and Lancaster 1970).

This flea is an important vector of the bacteria *Yersinia pestis* and *Rickettsia typhi*, the causative agents of plague and murine typhus, respectively (Durden and Hinkle 2009). Plague does not occur in Arkansas but human cases of murine typhus were recorded in the state in the first half of the 20th century until intensive domestic rat and flea control operations were implemented throughout the southern United States (Pratt and Good 1954). *Rickettsia typhi* may still circulate in enzootic transmission cycles between mammals and their ectoparasites in Arkansas as it does in some other southern states (Durden *et al.* 2012).

FAMILY RHOPALOPSYLLIDAE

***Polygenis gwyni* (C. Fox) – NCN.**

2M, 2F ex *D. virginiana*, Pulaski Co., North Little Rock, Camp Robinson, 30 Sept. 1943, coll: C.A. Hubbard (Smit 1987).

The hispid cotton rat (*Sigmodon hispidus*) is the most commonly recorded host of *P. gwyni* but there are also several records from *D, virginiana* and some other mammals throughout its range in the southern U.S. (Smit 1987, Durden *et al*. 2012). This flea is an inefficient vector of *R. typhi* (Pratt and Good 1954).

FAMILY CTENOPHTHALMIDAE

***Conorhinopsylla stanfordi* Stewart – NCN.**

1M, 1F ex 24 eastern fox squirrels (*Sciurus niger*), NW Arkansas, 1968 (Schiefer and Lancaster 1970).

This is a nidicolous flea of tree squirrels in eastern North America especially in northern U.S. states (and in Ontario, Canada) (Benton and Day 1980; Holland 1985; Eckerlin and Painter 1986). It has no known medical-veterinary importance.

***Conorhinopsylla nidicola* Jellison – NCN.**

1M ex 3 eastern woodrat (*Neotoma floridana*) nests, NW Arkansas, 1968 (Schiefer and Lancaster 1970).

This rarely encountered flea occurs in nests of *N. floridana* and was described from specimens collected in Kansas (Hopkins and Rothschild 1962). It has no known disease relationships.

***Corrodopsylla hamiltoni* (Traub) – NCN.**

1F ex 3 least shrews (*Cryptotis parva*), NW Arkansas, 1968 (Schiefer and Lancaster 1970).

1M ex *C. parva*, Benton Co., Bella Vista, W. Tanyard Hollow Rd., 9 Jun. 2016, coll.: K.G. Roberts (GSUENT L3807).

Cryptotis parva is the principal host of this flea which has been recorded mainly in the mid-western U.S. as far south as northcentral Texas (McAllister 1989). It has no known medical-veterinary importance.

***Ctenophthalmus pseudagyrtes* Baker – NCN.**

1M, 1F ex 20 *D. virginiana;* 4M, 7F ex 11 woodland voles (*Microtus pinetorum*); 2M, 2F ex 3 eastern moles (*Scalopus aquaticus*); 1M, 1F ex 3 *C. parva;* 1M, 1F ex 15 *S. hispidis*, NW Arkansas, 1968 (Schiefer and Lancaster 1970).

1F ex golden mouse (*Ochrotomys nuttalli*), Union Co., El Dorado, 10 Feb. 2013, coll: M.B. Connior (Tumlison *et al*. 2015) (GSUENT L3568).

1F ex *S. hispidus*, Marion Co., Mull, 9 Feb. 2015, coll: M.B. Connior (Tumlison *et al*. 2015) (GSUENT L3716).

1M ex southern short-tailed shrew (*Blarina carolinensis*), Union Co., El Dorado, 11 Feb. 2013 (Connior *et al*. 2014) (GSUENT L3569).

1M, 1F ex 2 *S. aquaticus*, Union Co., El Dorado, 7 & 8 May 2013, coll: M.B. Connior (Connior *et al*. 2014) (GSUENT L3588-L3589).

2F ex *S. aquaticus*, Union Co., El Dorado, 5 Sept. 2014, coll: M.B. Connior (GSUENT L3700).

1F ex *S. aquaticus*, Benton Co., Bentonville, 15 Feb. 2016, coll: M.B. Connior (GSUENT L3737).

Ctenophthalmus pseudagyrtes is mainly an ectoparasite of small mammals, especially Soricomorpha, in eastern North America as far west as Texas (Holland 1985; Durden *et al.* 2012; McAllister and Wilson 2012). It has no known medical-veterinary importance.

***Doratopsylla blarinae* Fox – NCN.**

1M, 1F ex *B. carolinensis*, Union Co., El Dorado, 28 Apr. 2013, coll: M.B. Connior (Connior *et al*. 2014) (GSUENT L3587).

1F ex northern short-tailed shrew (*Blarina brevicauda*), Searcy Co., 3 km S of Mull, 30 Aug. 2014 (Tumlison *et al*. 2015) (GSUENT L3701).

As reflected in the collection data reported here for Arkansas, this flea is associated with *Blarina* shrews and is widely distributed in eastern North America (Durden *et al*. 2012). It has no known medical-veterinary importance.

***Epitedia neotomae* Jameson – NCN.**

296M, 235F ex 3 *N. floridana* nests; 1F ex 3 *C. parva*; 2M ex 20 *D. virginiana;* NW Arkansas, 1968 (Schiefer and Lancaster 1970).

This flea mainly occurs in nests of *N. floridana* in the eastern and central U.S. (Hopkins and Rothschild 1962). It has no known disease relationships.

***Epitedia wenmanni* (Rothschild) – NCN.**

1M, 1F ex 2 *P. leucopus*, Marion Co., Mull, 18 Feb. 2013, coll: M.B. Connior (Tumlison *et al.* 2015) (GSUENT L3573 & L3578).

This is a flea associated with *Peromyscus* spp., and sometimes other rodents and their predators, across North America (Holland 1985; Durden *et al*. 2012). It has no known medical-veterinary importance.

***Rhadinopsylla fraterna* (Baker) – NCN.**

5M, 8F ex 3 *N. floridana* nests, NW Arkansas, 1968 (Schiefer and Lancaster 1970).

Rhadinopsylla fraterna mainly occurs in the Great Plains where it parasitizes ground squirrels, other rodents and, sometimes, their predators (Hopkins and Rothschild 1962; Holland 1985). It has no known medical-veterinary importance.

***Stenoponia americana* Baker – NCN.**

Ex: *P. leucopus* NW Arkansas, 1968 (Schiefer and Lancaster 1970).

1F ex *P. maniculatus*, 16 Mar. 1954, coll: J.P. Redman; 1M, 5F ex 5 *P. leucopus*, Jefferson Co., Jan.-Feb. 1955, coll: C.E. Hoffman (Hastriter *et al.* 2006) (NMNH, BZ-95, BZ-626, BZ-679, BZ-686).

This is the largest flea species in Arkansas and it mainly parasitizes *Peromyscus* spp. mice, some of the smallest mammals in the state. *Stenoponia americana* is widely distributed in eastern North America and Hastriter *et al*. (2006) also document a few records from the southwestern U.S. It is not known to transmit any pathogens or parasites.

FAMILY CERATOPHYLLIDAE

***Ceratophyllus celsus* Jordan – NCN.**

Ex northern cliff swallow (*Petrochelidon pyrrhonota*), Washington Co. (Baerg 1944).

5M, 5F ex *P. pyrrhonota* nests, NW Arkansas, 1968 (Schiefer and Lancaster 1970).

"Arkansas" (no other data) (Traub *et al*. 1983; Lewis and Galloway 2001).

This flea is a host-specific parasite of *P. pyrrhonota* and is mainly known from certain midwestern U.S. states (Traub *et al*. 1983). Hopla and Loye (1983)

suggested that *C. celsus* is a vector of an avian-associated trypanosome.

***Nosopsyllus fasciatus* (Bosc) – northern rat flea.**

Ex *R. norvegicus* and *R. rattus,* 21 localities across Arkansas (Pratt and Good 1954).

2F ex 17 *R. norvegicus*, NW Arkansas, 1968 (Schiefer and Lancaster 1970).

The northern rat flea can serve as an intermediate host for the rat tapeworm, *Hymenolepis diminuta*, a cosmopolitan worm that infects *Rattus* spp., but human infections are not uncommon. It can also transmit *R. typhi,* the causative agent of murine (flea-borne or endemic) typhus and the non-pathogenic kinetoplastid protist *Trypanosoma lewisi* from rat to rat (Durden and Hinkle 2009).

***Opisodasys pseudarctomys* (Baker) – NCN.**

1F ex southern flying squirrel (*Glaucomys volans*), NW Arkansas, 1968 (Schiefer and Lancaster 1970).

This flea is a host-specific ectoparasite of flying squirrels in eastern North America and is typically more common in the host nest (Benton and Day 1980, Eckerlin and Painter 1986). It has no known medical-veterinary importance but it could be an enzootic vector of North American strains of *Rickettsia prowazekii* (see comments below for *O. howardi*).

***Orchopeas howardi* (Baker) – NCN.**

3M, 11F ex 24 *S. niger*; 13M, 17F ex 26 eastern gray squirrels (*Sciurus carolinensis*); 1M, 2F ex 20 *D. virginiana*; 1F ex 3 *N. floridana* nests; ex *G. volans,* NW Arkansas, 1968 (Schiefer and Lancaster 1970).

6M, 19F ex 10 *P. lotor*, Van Buren Co., 1989–1990 (Richardson *et al*. 1994).

1M, 3F ex *G. volans*, Union Co., 11 km W of El Dorado, 5 Feb. 2013, coll: M.B. Connior (McAllister *et al*. 2013) (GSUENT L3549).

2M, 3F ex *G. volans,* Union Co., El Dorado, 5 Feb. 2013, coll: M.B. Connior (McAllister *et al*. 2013) (GSUENT L3560).

1M, 1F ex *S. niger*, Marion Co., Mull, 23 Dec. 2012, coll: M.B. Connior (McAllister *et al*. 2013) (GSUENT L3550).

1M, 2F ex 2 *S. carolinensis,* Marion Co., Mull, 23 Dec. 2012, coll: M.B. Connior (McAllister *et al.* 2013) (GSUENT L3551-L3552).

2F ex 2 *S. carolinensis* nests, Union Co., El Dorado, 7 & 9 Feb. 2013 (McAllister *et al*. 2013) (GSUENT 3570-3571).

Lewis (2000) designated a female *O. howardi* (host not reported) from Little Rock, Pulaski County, as the source of the female diagnostic characters for this species. Lewis (2000) also noted that *O. howardi* had been found on humans, though usually singly, but he did not know of published records of its feeding. This flea can transmit North American strains of *R. prowazekii*, the causative agent of sporadic epidemic typhus which is maintained enzootically in flying squirrel populations (McDade 1987). Serologically confirmed human cases of this disease have been recorded in Arkansas (McDade 1987).

***Orchopeas leucopus* (Baker) – NCN.**

1F ex *P. leucopus*, Marion Co., Mull, 18 Feb. 2013, coll: M.B. Connior (Tumlison *et al*. 2015) (GSUENT 3577).

4M, 4F ex 3 Texas mice (*Peromyscus attwateri*), Searcy Co., 3 km S of Mull, 19 Jan. 2015, coll: M.B. Connior (Tumlison *et al*. 2015) (GSUENT L3717–L3719).

1M ex *P. attwateri*, Carroll Co., NE of Berryville, 14 May 2016, coll: M.B. Connior (GSUENT L3799).

1M, 1F ex *P. maniculatus*, Benton Co., NE of Maysville, 21 May 2016, coll: M.B. Connior (GSUENT L3800).

Orchopeas leucopus occurs across North America mainly as an ectoparasite of *Peromyscus* spp. mice, although there are records from other mammals (Durden *et al*. 2012). It has no known medical-veterinary importance.

***Orchopeas pennsylvanicus* (Jordan) – NCN.**

141M, 190F ex 3 *N. floridana* nests; 1F ex *P. leucopus*, NW Arkansas, 1968 (Schiefer and Lancaster 1970).

Schiefer and Lancaster (1970) reported this flea as "*Orchopeas sexdentatus* subsp." implying *O. sexdentatus pennsylvanicus* which was the name for the previously recognized subspecies that parasitizes *N. floridana.* Lewis (2000) elevated this subspecies to full species status. It occurs in eastern North America as an ectoparasite of woodrats (Lewis 2000) and has no known medical-veterinary importance.

FAMILY LEPTOPSYLLIDAE

***Odontopsyllus multispinosus* Baker – NCN.**

1F ex 2 *D. virginiana;* 1F ex 7 *S. floridanus;* 1F ex 2 *U. cinereoargenteus;* NW Arkansas, 1968 (Schiefer and Lancaster 1970).

This is a large flea associated with leporids and their predators in eastern North America (Holland 1985, Durden *et al*. 2012). It has no known medical-veterinary

importance but, based on data presented by Hopla (1980a), it could be an enzootic vector of *F. tularensis.*

***Leptopsylla segnis* (Schönherr) – European mouse flea.**

Ex *R. norvegicus* and *R. rattus,* 10 localities across the southern two-thirds of Arkansas (Pratt and Good 1954).

The European mouse flea is distributed across North America as an ectoparasite of the house mouse (*Mus musculus*) and of peridomestic *Rattus* spp. (Durden *et al*. 2012). However, it appears to be less common in the U.S. than it was during the first half of the 20th century when intensive rat and rat-flea elimination programs were widely implemented, especially in the southern U.S. (Pratt and Good 1954). This flea is an inefficient vector of the causative agents of murine typhus and plague (Durden and Hinkle 2009).

***Peromyscopsylla scotti* I. Fox – NCN.**

Ex *P. leucopus*, NW Arkansas, 1968 (Schiefer and Lancaster 1970).

Peromyscopsylla scotti mainly parasitizes *Peromyscus* spp. in the eastern U.S. and there are previous records from Kansas and Oklahoma (Holland 1985; Durden *et al*. 2012). It has no known medical-veterinary importance.

FAMILY ISCHNOPSYLLIDAE

***Nycteridopsylla chapini* (Jordan) – NCN.**

1M ex big brown bat (*Eptesicus fuscus*), Benton Co., Indian Cave, 19 Jan. 1941, coll: E. Crawley (Sanderson 1941; Lewis 1957; Lewis and Wilson 1982).

1F ex *E. fuscus,* Madison Co., Mitchell Cave, 26 Feb. 1955, coll: J.A. Sealander (Lewis and Wilson 1982).

Nycteridopsylla chapini is an ectoparasite of bats, especially *E. fuscus,* in the eastern and midwestern United States (Lewis 1957; Lewis and Wilson 1982). Examination of numerous *E. fuscus* by CTM in July 2002 from Cushman (Blowing) Cave, Independence County, Arkansas, did not find this flea. It has no known medical-veterinary importance.

FAMILY VERMIPSYLLIDAE

***Chaetopsylla lotoris* (Stewart) – NCN.**

15M, 24F ex 9 *P. lotor,* Van Buren Co., 1989–1990 (Richardson *et al*. 1994).

This flea is a specific parasite of *P. lotor* in eastern North America from Maine to North Carolina westward to Ontario and Arkansas (Holland 1985, Richardson *et al*. 1994). It has no known medical-veterinary importance.

UNIDENTIFIED ARKANSAS FLEAS

Caster *et al*. (1994) reported that 80% of nest boxes used by *G. volans* in Garland County of the Ouachita Mountains harbored fleas. To our knowledge, unfortunately, these fleas were not identified; therefore, records are not placed in that county on Fig. 1. Benton and Day (1980) and Eckerlin and Painter (1986) reported 4 common species of fleas infesting *G. volans* and their nests in New York, Vermont and Virginia, namely *O. howardi, C. stanfordi*, *Epitedia faceta*, and *O. pseudarctomys*. All of these species except *E. faceta* are also reported in this paper from Arkansas and 2 of these (*O. howardii* and *O. pseudarctomys*) were collected from *G. volans*.

Discussion

We have provided a synopsis of the 29 species of fleas recorded from Arkansas, an increase of 8 species over the work of Schiefer and Lancaster (1970). We suggest that, in addition to examination of animals for fleas, examination of their nests is also warranted, for the possibility of recording additional fleas of the state. The majority of flea records have been reported for counties in the Ozark Mountains physiographic region (Fig. 1). However, not all records reported herein could be placed on Fig. 1 because those for 4 species of fleas (*X. cheopis*, *N. fasciatus*, *E. gallinacea*, *L. segnis*) were not specified in a previous report (Pratt and Good 1954). Therefore, additional surveys are warranted for counties in other physiographic regions in eastern, western, and southern Arkansas.

Several of the flea species recorded here in the state have medical-veterinary importance. In addition to the flea-borne pathogens and parasites mentioned in the species accounts, some fleas such as *C. felis,* are nuisance biters. Some pets and humans are hypersensitive to flea bites and develop flea bite allergies that lead to intense pruritus (itching), scratching, dermatitis, and the possibility of secondary bacterial infections (Durden and Hinkle 2009). There is circumstantial evidence that rodent associated fleas transmit *Bartonella* spp. and other bacteria to their hosts (Abbott *et al*. 2007). Fleas in Arkansas may also transmit *F. tularensis, Coxiella burnetii* (the causative agent of Q fever) and other microorganisms such as certain rickettsial bacteria, especially in enzootic transmission cycles involving wild mammals (Durden and Hinkle 2009).

Acknowledgments

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Simulating Foodborne Pathogens in Poultry Production and Processing to Defend Against Intentional Contamination

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Running Title: Simulating Pathogens in Poultry Production

Abstract

There is a lack of data in recent history of food terrorism attacks, and as such, it is difficult to predict its impact. The food supply industry is one of the most vulnerable industries for terrorist threats while the poultry industry is one of the largest food industries in the United States. A small food terrorism attack against a single poultry processing center has the potential to affect a much larger human population than its immediate consumers. In this work, the spread of foodborne pathogens is simulated in a poultry production and processing system to defend against intentional contamination. An agent-based simulated environment that represents the farm, processing plant, homes, and restaurants is developed, which contains both poultry and human agents that move through the system and possibly infect each other. The simulation is run by varying several parameters that include probability of infection if exposed for both poultry and humans. The simulation predicts the number of infected poultry and humans over time.

Introduction

Often overlooked as a contingency, the food supply sector represents a substantial risk in human safety and healthy lifestyles. While safe transportation and regulation is being pursued heavily after the events of September 11, 2001, there is considerable uncertainty in the ability to prevent or halt food terrorism, defined as "an act or threat of deliberate contamination of food for human consumption with biological, chemical, and physical agents or radionuclear materials for the purpose of causing injury or death to civilian populations and/or disrupting social, economic, or political stability" (Setola and Maggio 2009). Tommy Thompson, the Secretary of the Department of Health and Human Services, even hinted toward the unpreparedness of the United States in regard to food terrorism when he resigned, stating, "I, for the life of me, cannot understand why the terrorists have not . . . attacked our food supply because it is so easy to do" (Roberts 2006).

There is a lack of data for intentional contamination and possible outcomes due to lack of actual attacks making it past the initial target; however, a biological attack has potential to affect a larger population as a whole. This lack of data makes preparing for food terrorism difficult (Layfield *et al*. 2008).

The top three most important foodborne outbreaks of 2016 include *Salmonella* linked to poultry, *Listeria* linked to frozen vegetables, and hepatitis A from raw scallops (Flynn 2016). CDC's FoodNet monitors foodborne diseases from ten United States cities and in 2016 identified 24,029 infections, 5,512 hospitalizations, and 98 deaths caused by foodborne pathogens (Marder *et al*. 2017). The FoodNet surveillance network does not track all cases in the United States (CDC 2017) and the most recent estimate of the total number of cases is from a 2011 study (Scallan *et al*. 2011). Foodborne morbidity and mortality associated with pathogen contamination of the United States food supply results in an estimated 48 million cases, of which 128,000 are hospitalized and 3,000 are fatal (Handley *et al.* 2015; Scallan *et al*. 2011). This estimation means that approximately 15% of the United States population is affected with a foodborne illness every year. Of all these illnesses, salmonellosis is one of the most common, costing $3.3 billion annually in medical bills and productivity loss in the United States (Handley *et al*. 2015). These are most likely not intentional contaminations, but it begins to shine some light on how vulnerable the industry could be if an intentional attack slipped through the cracks.

Poultry products rank in the upper echelon of commonly consumed foods, globally, and in the United States, poultry began surpassing beef consumption after 2010 (Handley *et al*. 2015). In 2013, the United States

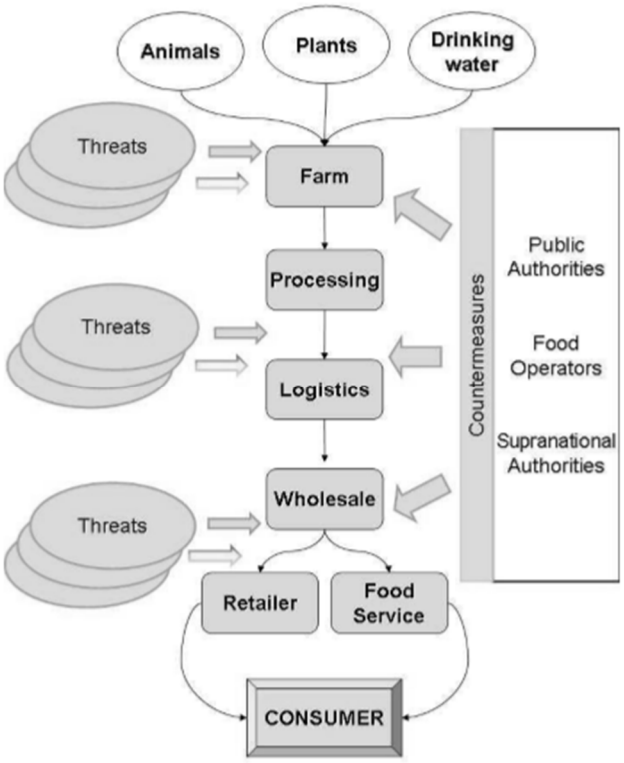

Figure 1: The general poultry food supply chain (Setola and Maggio 2009).

measured in at 639.6 million pounds of broiler meat shipped (Handley *et al*. 2015). As one of the largest sources of food in the United States, poultry is a top contender for possible food terrorism targets. There are also many vulnerable entry points for threats between each processing step as shown in Figure 1.

Even if a foodborne illness threat is neutralized quickly, traced back to the source, and taken off the shelves, if there were some people affected, there is still the possibility for contagious varieties of pathogens to be passed around to other people.

Methods

Overview

The approach taken in this project is to simulate the spread of foodborne pathogens among poultry and humans using an agent-based simulation model. The simulation steps are: use a focused software suite specifically for agent-based simulation, choose common and substantial pathogens to simulate, and determine agents such as chickens and humans.

The software suite chosen for this project is NetLogo, a robust modeling environment for designing agent-based simulations (Wilensky 1999). In NetLogo, each agent is programmed with a set of rules for actions such as movement around patches and interactions with other agents. It comes with disease models (Rand and Wilensky 2008) and has been used for modeling the immune system (Chiacchio *et al*. 2014).

In the United States, it is estimated that 31 different pathogens end up causing 37.2 million morbidity and mortality with 9.4 million of them being foodborne. *Salmonella* is one of the most common pathogens in the United States at 1 million estimated annual morbidity and mortality cases, 19,000 estimated annual hospitalizations, and 380 estimated annual fatal cases (Scallan *et al*. 2011). As prominent as it is, *Salmonella* was chosen as a starting point for gathering meaningful simulation data. The Center for Disease Control and Prevention (CDC) would be considered a good primary resource for further pathogen selection.

Having a software suite and pathogen to study is only half of the simulation: the simulation also requires the interacting agents, for example, poultry and humans in the current case. The simulation distinguishes different demographics in the humans, as there are varying susceptibilities to *Salmonella* and other pathogens. For example, the age of a given population will affect how easily the illness affects the agent. In addition to the varying demographic, the project manipulates the infection rate based on how much exposure to the food pathogen sources occurs when they are being consumed. For example, it is necessary to consider a specific population's frequency in eating out of home to adjust the exposure of certain pathogens. Human agents were divided into three age groups: young, middle, and old based on differing susceptibility to the given pathogen, *Salmonella*.

During the different parts of production, as shown in Figure 1, the poultry have multiple opportunities to encounter the pathogen. As they get further along the supply chain, through processing, logistics, and consumption, the poultry are moved around in groups (not autonomously roaming) and may come into contact with other poultry who in turn may also become infected. As the poultry are moved to wholesalers, stores, or restaurants, they may come in contact and infect humans based on exposure to the infected poultry.

NetLogo Overview

NetLogo identifies various groups of agents with their individual behaviors and frees them to disperse and engage in an interactive environment (Wilensky 1999). Simulations are comprised of turtles, the moving and

acting agents in the simulation, and patches, the space in which the turtles move and interact.

The turtles are sectioned into differing breeds that have different rules and variables to act under. These different breeds then move around and can be set to behave in specified ways depending on what breed with which they are interacting.

The patches act as a grid that the turtles are set to move around and possibly interact with other turtles. Each patch can have different properties that affect turtles and perhaps other patches.

Every time tick, there is a loop that goes through each turtle and tells them to do their next step in the simulation. The ticks can represent any appropriate unit of time such as seconds, minutes, hours, or days. Ticks can be slowed down or sped up to focus on specific areas of the simulation or to generally speed things up to gather a greater quantity of data.

Breeds, Patch Types, and Customizable Properties

For this project, there are 2 different breeds of turtles and 4 different kinds of patches. Turtles can be either poultry (plural poultry) or person (plural people) as shown in Figure 2. Both breeds may also be gray, signifying a pathogen infection, or black, indicating no infection. There are four different kinds of patches representing the farm, processing plan, restaurants, and houses.

Both the person breed and poultry breed have a member variable for infection. When true, the person or poultry will change from its normal color variation (black) to its infected color (gray). There is also an infection modifier variable set upon turtle creation that can manipulate the probability for that person/poultry to be infected. The infection modifier mostly comes into play for differing age groups of people since there are varying susceptibilities to pathogens.

The poultry breed has properties to help identify which part of the supply chain it should be in currently. There is a counter variable to keep track of how long it has been in its current section. There are also two Boolean properties, alive and processed, to identify which sections the poultry have already visited. If the poultry are not alive, then they have already been slaughtered, etc.

The person breed has four separate properties: age group, infection timer, house number, and restaurant timer. The age group property determines the turtle property infection modifier. People have an adjustable infection timer to specify how long they are infected with pathogens such as *Salmonella* that are typically fought off after a week's time. The house number is the number of the house to which each person is assigned. The restaurant timer is for counting down how long a person has been in a restaurant.

Figure 2: Poultry and persons colored black indicate no infection, while poultry and persons colored gray represent a pathogen infection

The four different patch types do not act by themselves, but they do affect the actions of the turtles on them. Turtles check the kind of patch they are on and act accordingly. For instance, when on the farm patch, the poultry breed roams around randomly. While on the processing patch, the poultry stay in the position they are assigned. Both breeds stay stationary on the restaurant patch. The person breed stays stationary while on the house type. The farm patch type includes a large area to allow the poultry to move around freely. The processing patch type also includes an area, although it is much smaller than the farm type. The restaurant and house patches are setup to be individual patches that count the number of people currently in that patch.

In addition to all the specific properties for turtles, there are a variety of sliders easily changed in the user interface. These sliders include the following: setting the number of people in the simulation, the number of houses and restaurants, the frequency people visit restaurants, the infection duration, the probability of poultry infecting people on the same patch or poultry on the same patch, the initial number of poultry, and the spawn rate of poultry.

Workflow

The simulation is loosely based off Figure 1 and the simulation flow diagram is shown in Figure 3, with the poultry trickling down through steps where threats can be inserted, finally landing in a patch with the

consumers. Prior to the simulation starting, or any time during the simulation, the user can select poultry to "get-infected". This is how intentional contamination is simulated.

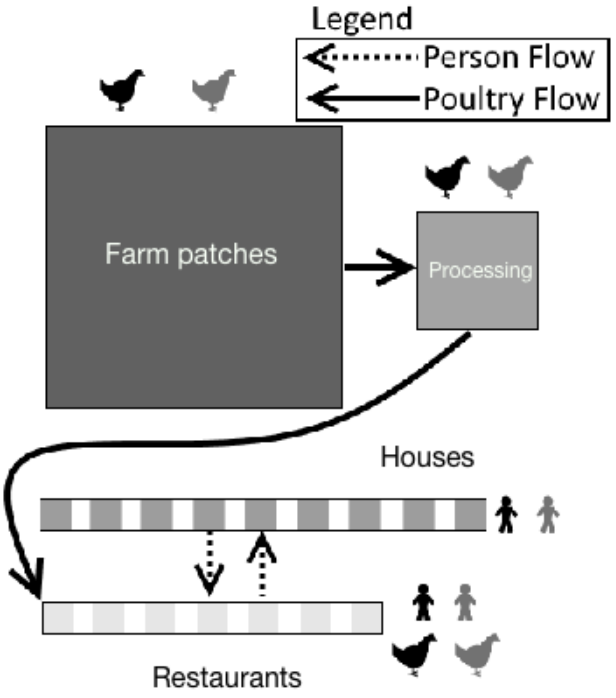

Figure 3: Simulation flow diagram of the flow of poultry and persons in the simulated environment consisting of the farm, processing plant, restaurants, and houses. See Figure 2 for legend.

When the simulation is initiated, there is a set number of poultry provided by a slider. These poultry are placed in the large farm patch section. There is also a spawn rate for poultry to be continuously added to the farm patch section to simulate continual poultry breeding. Each poultry has a timer and, when it reaches a threshold, it moves to the next section. This timer is meant to simulate a poultry's growth cycle before being butchered. During its time in the farm patch section, each simulation tick, poultry randomly select a direction around them in a 360-degree radius and move forward one patch. If there is an infected poultry on a given patch, there is a probability, modified by slider, for other poultry on the given patch to also become infected.

The second section poultry move to after their counter is expired is the smaller processing patch section. Unlike the farm patch section, once a poultry is assigned a specific patch in the processing plant patch section, the poultry does not move. Multiple poultry can be placed on one patch. This is meant to represent groups of poultry being close together during the processing stage while not really being in contact with some other groups. If there is a poultry on a given patch that is infected, there is a probability of infecting other poultry on the same patch at each simulation tick. A new counter is started for each poultry when moved to the processing patch section.

The third and final section for poultry is the restaurant. After a poultry's processing plant section timer reaches a threshold, the poultry is moved to a randomly selected restaurant. A final countdown is started once moved to a restaurant, and the poultry is deleted at the end of this timer to simulate the poultry being consumed. If there is an infected poultry in a restaurant patch, there is a probability every tick that any poultry or person in that restaurant patch will also become infected.

The person turtles simply alternate between the house patches and the restaurant patches. An initial number of people is set before the simulation setup and the number of people never changes throughout the simulation. When a person is created, it is assigned a house patch to which it will always return. While on a house patch, people can be set to have a chance to infect the other people in the house, or the slider can be moved all the way to make 0% of people infecting each other.

Every tick, there is a probability, set by slider, that each person will go to a randomly selected restaurant patch. These are the same restaurants that poultry can be sent to during their final step. If there is an infected poultry in a restaurant, it has a probability of infecting the person that has arrived at the restaurant. This is the driving interaction of people becoming infected from the infected food supply. If people are set to be able to infect each other, a person may become infected by another person visiting the restaurant. The amount of time that people stay in restaurants can be set by slider and adjusted to better simulate the shorter duration of restaurant visit and longer duration of staying at home.

Results

The developed simulations can visualize and quantify multiple scenarios with varying parameters. For example, a plot that shows the number of uninfected (healthy) people along with the number of infected people with three infection rates is shown in Figure 4 and a plot that shows the number of uninfected poultry along with the number of infected poultry with three infection rates is shown in Figure 5. Both plots update every tick in the simulation and can easily be exported to a spreadsheet to conduct further analysis.

Figure 4 shows three different sections of time that had differing infection probabilities in people. The section with the line labeled with a "1" shows a 0.1% poultry-to-people infection probability per tick, section "2" shows a 2.5% infection probability, and section "3" shows a 5.0% infection probability. The data changes in real time as adjustments are made to the simulation sliders. It is clear to see that the difference between 1% and higher percentages is strong while the doubling from 2.5% to 5.0% makes a much smaller difference

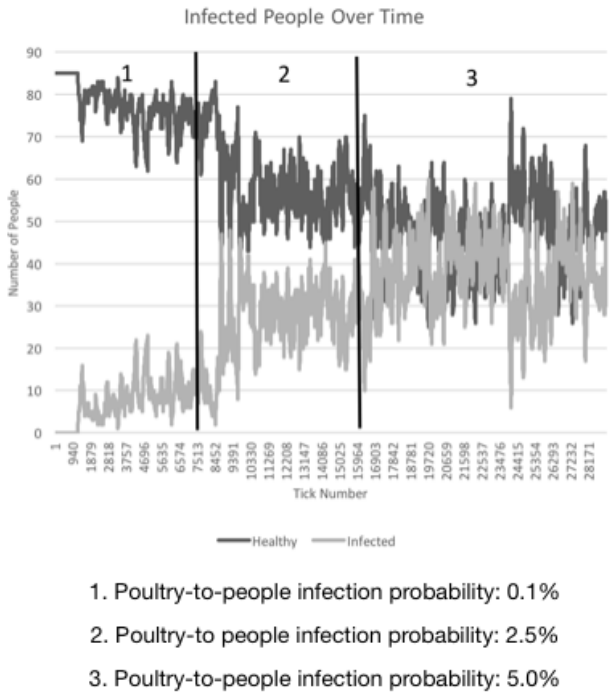

Figure 4: Number of infected people over time with different poultry-to-people infection probabilities

Figure 5 shows the number of poultry at three different periods of time that had differing poultry-to-poultry infection probabilities. Section "1" shows a poultry-to-poultry infection probability of 5.1%, section 2 shows a 10% probability, and section 3 shows a 30.05% probability. The sections over 5.1% show a significant increase in infection. While 10% and 30.05% probabilities do not differ much in terms of maximum amount of poultry infected at one time, 30.05% probability shows a much less varied graph.

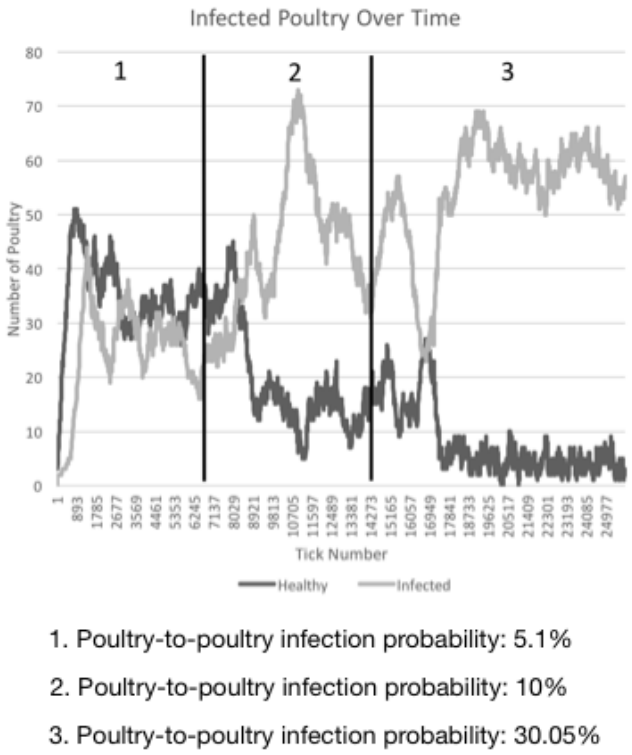

Figure 5: Number of infected poultry over time with different poultry-to-poultry infection probabilities

Conclusions and Future Work

A food supply chain intentional pathogen injection simulation was built using the NetLogo agent-based modeling simulation software for a poultry production and processing system. These simulations can help prevent food terrorism by predicting the spread and effect of foodborne pathogens including the number of infected poultry and the number of infected people over time with varying probabilities of infection. The simulation is loosely based on the poultry food supply chain, but it can be improved in the future by adding more stages in the production and processing, simulating the use of antibiotics and cleaning methods, and by using more accurate epidemiological models to create a more realistic simulation of the system. In addition, another category of highly susceptible people such as cancer patients on chemotherapy could be added. It would be interesting to compare and contrast an actual paired set of demographics for example a suburban Florida community with more retirees compared to an inner-city area with younger people. Finally, once a more detailed model is developed, it could be validated by comparing it with an actual well-documented outbreak.

Acknowledgements

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Long-term Aquatic Invertebrate Monitoring at Buffalo National River, Arkansas

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Running title: Aquatic invertebrate monitoring at the Buffalo River

Abstract

Aquatic invertebrate community structure was used to assess long-term water quality integrity in the mainstem of the Buffalo National River, Arkansas from 2005 to 2013. Nine benthic invertebrate samples were collected from each of six sampling sites using a Slack-Surber sampler. The Stream Condition Index (SCI) developed for Ozark streams was used to assess integrity of the invertebrate communities. This index is calculated using taxa richness, EPT (Ephemeroptera, Plecoptera, Trichoptera) Richness, Shannon's Diversity Index, and Hilsenhoff Biotic Index (HBI). Sørensen's similarity index was used to assess community similarity among sites, and scores were then analyzed using ascendant hierarchical cluster analysis. The benthic invertebrate fauna was diverse with 167 distinct taxa identified from all sites, with similarities ranging from 70% to 83%. Cluster analysis showed that sites were clustered in a longitudinal progression, with those sites closest to one another in linear distance generally being the most closely related. Overall, the invertebrate taxa of the Buffalo River are largely intolerant (mean tolerance value= 4.38). Taxa richness was typically greater than 20 among samples, and EPT richness values consistently were greater than 12 for all sites in most years. Shannon's diversity index values generally ranged from 2.0 to 2.5 among sites and years. Metric values tended to decrease in a downstream direction to Site 4, and then increase to levels observed upstream. The exception was for HBI, which did not show this response and values for this metric generally were below 5. SCI scores among sampling sites were variable but not generally impaired and were fully biologically-supporting. Water quality (temperature, dissolved oxygen, specific conductance, pH, turbidity) met state standards in all instances. Habitat data were summarized, but found to be poorly correlated with invertebrate metrics (<30% significant). Although the condition of invertebrate communities and water quality in the Buffalo River are largely sound and have high integrity, numerous ongoing and projected threats to these resources remain, and those threats largely originate outside of the park's jurisdictional boundaries. Inherent variability of invertebrate community diversity and density across sites and years highlights the importance of using multi-metric assessment and multiyear monitoring to support management decisions.

Introduction

The Buffalo National River (BUFF) was established in 1972 to protect the corridor of the Buffalo River and its tributaries. However, the NPS jurisdictional boundary of the Buffalo River is generally a narrow corridor that encompasses only about 11% of the watershed, while over 60% of the watershed is in private ownership (Mott and Luraas 2004). This leaves much of the watershed unprotected from human activities such as timber management, landfills, grazing, livestock operations, urbanization, gravel mining, stream channelization, and removal of riparian vegetation. Wadeable streams of the Ozarkian region, including those at BUFF, generally are in relatively good condition, but the previously noted stressors threaten their integrity (Petersen and Femmer 2002; Petersen 2004; Huggins *et al*. 2005; United States Environmental Protection Agency 2006). Since the establishment of BUFF, more of the watershed has been deforested than is protected within the boundaries of the National River (Scott and Hofer 1995; Scott and Udouj 1999; Mott 2000). This is problematic because land use practices at the watershed level tend to overwhelm localized protection of stream corridors (Roth *et al*. 1996; Heino *et al*. 2003; ZumBerge *et al*. 2003). For example, increases in bank erosion rates and changes in channel morphology through time have been correlated with increased land clearing of steep uplands within a stream basin (Stephenson and Mott 1992; Jacobson and Primm 1997), as well as historical riparian land clearing (Panfil and Jacobson 2001). Moreover, the Buffalo River basin is located in an area of extensive karst topography,

making its streams vulnerable to contaminated groundwater recharge and interbasin transfer of groundwater from adjacent watersheds (Brahana *et al*. 2016; Watershed Conservation Resource Center 2017). Although all new discharges to the catchments of the Buffalo River are prohibited as part of an anti-degradation strategy (United States Code of Federal Regulations 2012), historical and ongoing pollutant discharges remain (Hovis 2014; Usrey 2013; Brahana *et al*. 2016; Watershed Conservation Resource Center 2017). Protecting and maintaining the integrity of the natural resources of the Buffalo River is a high priority because this river also serves as a major economic contributor to the region largely through tourism and park visitation (Cui *et al*. 2013; Cullinane *et al*. 2014).

Aquatic invertebrates are an important tool for understanding and detecting changes in ecosystem integrity, and they can be used to reflect cumulative impacts that cannot otherwise be detected through traditional water quality monitoring (Barbour *et al*. 1999; Moulton *et al*. 2000, 2002). Benthic community structure can be quantified to reflect stream integrity in several ways, including the occurrence of pollution sensitive taxa, dominance by a particular taxon combined with low overall taxa richness, or appreciable shifts in community composition relative to a reference condition (Lazorchak *et al*. 1998; Barbour *et al*. 1999; Bonada *et al*. 2006).

Stream assessments using aquatic invertebrates are typically short-term, single events aimed at assessing stream integrity for a given section of stream in relation to stressors such as bacterial or chemical pollution, and habitat disturbance. By comparison, long-term monitoring at fixed, permanent sites is much less common. Such long-term monitoring is particularly important because the variability over time of metrics used in bioassessments has been shown to be high in other studies (Bruce 2002; Jackson and Füreder 2006; Mazor *et al*. 2009; Vaughan and Ormerod 2012; Bowles *et al*. 2013a, 2013b). Evaluation of long-term variability helps researchers and managers better understand alterations in stream condition relative to climatic variability and change, as well as other anthropogenic disturbances (Jackson and Füreder 2006; Vaughan and Ormerod 2012).

There have been several previous studies conducted on stream invertebrate communities at BUFF for the purpose of assessing water quality impacts and ecological integrity (see Bowles *et al*. 2007 for review). They include Kittle (1975), Geltz and Kenny (1982), Bryant 1997, Mathis (1990, 2001), Mott (1997), Radwell (2000), and Usrey (2001). All of these works exist as gray literature and have not been published. Additionally, these studies were based on either single season events, or multiple season events within the same year. Other aquatic invertebrate studies at BUFF have attempted to take a more comprehensive and long-term approach to assessing invertebrate community dynamics and stream integrity. For example, Mathis (2001) developed an Index of Community Integrity (ICI) for the Buffalo River based on multiple metrics from seasonal collections within the river basin.

The National Park Service's Heartland Inventory and Monitoring Network (HTLN) began monitoring at BUFF in 2005. Bowles *et al*. (2007) included the ICI in the original monitoring protocol to assess long-term aquatic invertebrate community structure at fixed, randomly selected sites at BUFF and directed towards maintaining the ecological integrity of the river and its tributaries. Subsequently, the ICI was not selected for further use because it was judged inferior to the simpler Stream Condition Index (SCI) developed for neighboring Missouri (see DeBacker *et al*. 2012). Bowles *et al*. (2013c) presented a summary of the first few years of this monitoring program. A previous study addressed aquatic invertebrate communities in BUFF tributaries (Mixon-Hinsey 2008).

Here, the results of monitoring aquatic invertebrate community structure and habitat at permanent mainstem Buffalo River sites conducted from 2005 to 2013 are summarized.

Methods and Materials

Site Selection

Sampling was conducted at 6 permanent mainstem river sites on the Buffalo River annually from 2005 to 2009, and again in 2011 and 2013 (Fig. 1). See Bowles *et al*. (2007) for a description of site selection and supporting data. All samples were collected from riffles during a November through February index period with most samples being collected during December and January. Site 1 was dry during the index period in 2005 and could not be sampled, and in 2006 Site 6 was flooded during most of the index period and also could not be sampled.

Aquatic Invertebrates

Three benthic invertebrate samples were collected from each of three successive riffles at each sampling site using a Slack-Surber sampler (500 μm mesh, 0.25 m^2, n=9). The sample area was agitated for 2 minutes with a garden cultivation tool, and large pieces of substrate were scrubbed with a brush as necessary to

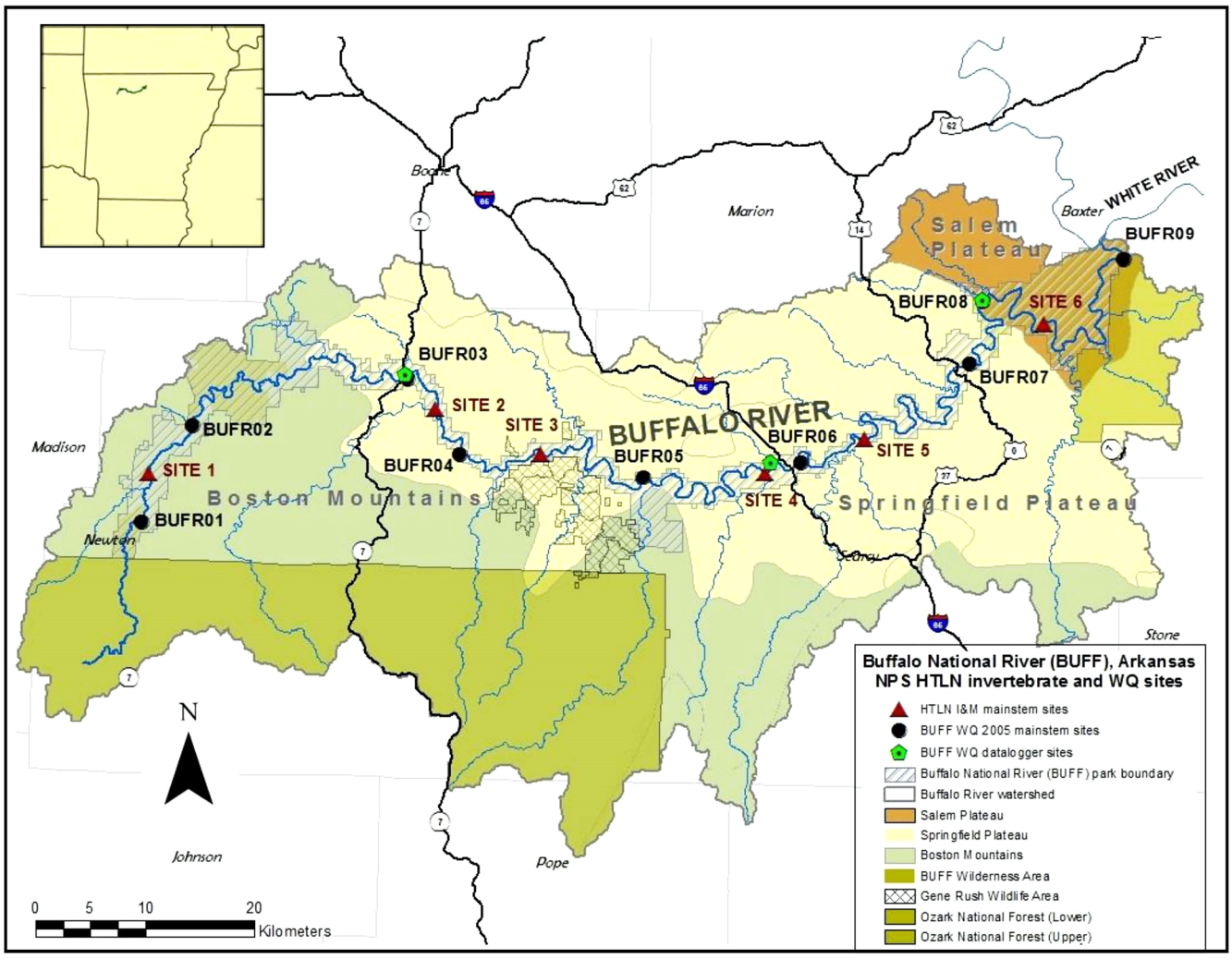


Figure 1. Location of water quality and benthic invertebrate sampling sites on the Buffalo River. BUFF water quality sampling locations are black circles, HTLN monitoring sites are red triangles, and data logger sites are green pentagons.

remove attached invertebrates. Samples were placed in plastic jars and preserved with either 99% isopropyl or 95% ethyl alcohol. Samples were sorted in the laboratory following a subsampling routine described in Bowles *et al*. (2007), and taxa were identified to the lowest practical taxonomic level (usually genus) and counted.

In addition to sampling conducted by the HTLN, BUFF natural resources staff collected invertebrate samples from nine mainstem Buffalo River water quality sites during a BUFF water quality bioassessment study in 2005 (Fig. 1). The data from that study are maintained in the HTLN database (HTLN 2016). Collection methods used by BUFF staff were analogous to those reported here and the data can therefore be directly compared. Data from that study are analyzed in this report for the purpose of comparison to our monitoring sites and data to provide a broader picture of invertebrate community structure and integrity.

Multi-metric Index

The Stream Condition Index (SCI), a multi-metric index developed by Rabeni *et al*. (1997) for the state of Missouri, was used to assess integrity of invertebrate community data. The SCI is a multi-metric index founded on data collected from 26 reference streams in the Ozarks region (Rabeni *et al*. 1997). This index is calculated using four metrics as measures of community structure and balance, including taxa richness, EPT (Ephemeroptera, Plecoptera, Trichoptera) richness, Shannon's diversity index, and Hilsenhoff Biotic Index (HBI; Hilsenhoff 1982, 1987, 1988). High values are preferred for all metrics, except for HBI, where smaller values are the desired response. An increase in HBI

values over time is undesired, because that would reflect the community's increasing tolerance to disturbance. See Bowles *et al*. (2007) for sources of assigned tolerance values. The chosen metrics are sound measures of community structure and balance and are generally considered sufficiently sensitive to detect a variety of potential pollution problems in Ozark streams (Rabeni *et al.* 1997) (Table 1). All metric values used are normalized so that they become unitless and can be compared, and have equal influence on the SCI results. The lower or upper quartile of the distribution for each metric is used as the minimum value representative of reference conditions (Table 1). Mean metric values were established by averaging the values for each of three samples per riffle and then averaging the means for the three riffles to establish a site mean (n=3). Procedures for calculating and scoring these four metrics and the SCI can be found in Bowles *et al*. (2007) and Sarver *et al*. (2002). The SCI produces three possible levels of stream condition: 1) fully biologically supporting (unimpaired), 2) partially biologically supporting (impaired), and 3) non-biologically supporting (very impaired). Unimpaired or reference sites score ≥16 and have the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of the natural habitat of the region. Both partially biologically supporting (SCI 10-14) and non-biologically supporting (SCI 4-8) categories indicate impaired streams that do not meet the beneficial use of protection of aquatic life.

Habitat and Water Quality Assessment

Dominant substrate was visually estimated from three randomly selected pieces within the sampling net frame using the Wentworth scale (Wentworth 1922). Depth (cm) and current velocity (m/sec) were measured immediately in front of the sampling net frame using a top-setting wading rod fitted with a Marsh-McBirney Flow-Mate 2000 flow meter. Qualitative habitat variables (percent substrate embeddedness, periphyton, filamentous green algae, and aquatic vegetation) were estimated within the sampling net frame as percentage categories (0, <10, 10-40, 40-75, >75). Habitat data were analyzed as midpoints of each category across years for each site.

Static readings of water quality parameters (temperature, dissolved oxygen, specific conductance, pH) were recorded at each riffle sampled with calibrated, hand-held instruments (YSI models 55, 63, ProPlus). In addition, hourly readings of water quality parameters (temperature, dissolved oxygen, specific conductance, pH, turbidity) were recorded continuously at 1 hour intervals at least 1 week prior to sampling using calibrated data loggers (YSI models 6600, 6920) at three fixed sites on the Buffalo River located near Site 2, Site 4, and between Site 5 and Site 6 (Fig. 1). The water quality data collected for this study are only intended to describe the prevailing conditions that may influence the structure of invertebrate communities, and they represent only a small snapshot of the broader range of possible conditions over longer periods. Due to the limitations of using water quality data obtained with data loggers, the invertebrate community is used here as a surrogate of long-term water quality conditions. Water quality data are summarized across years and presented as single means to represent each site.

Statistical Analysis

Sørensen's Similarity Index (presence/absence) was used to analyze similarity of taxa occurrences among the different sampling sites (Southwood and Henderson 2000; Hammer *et al*. 2001). Similarity index scores among sites were analyzed using ascendant hierarchical cluster analysis (Ward 1963) following the recommendation of Magurran (2004).

Pairwise correlation coefficients for each pair of metrics and habitat variables were calculated using nonparametric Kendall's tau (Daniel 1990) because examination of histograms revealed lack of normality for many of the habitat variables. This analysis evaluated correlations between the four biological metrics calculated from aquatic invertebrate samples and 11 habitat variables. The habitat variables included: embeddedness, vegetation, periphyton, algae, depth, velocity, substrate size, dissolved oxygen, temperature, specific conductance, and pH. Data were grouped separately and analyzed by year and by site. When grouped by year, all riffles from all sites were included in the same analysis, and the analysis was repeated for each year (N = 7 years; n = 18 observations for each correlation: 3 riffles x 6 sites) (4 metrics x 11 habitat variables x 7 years = 308 total correlations). This approach provided the strongest level of independence among observations. When grouped by site, all years of data for all riffles of each site were included, and the analysis was repeated for each site (N = 6 sites; n = 21 observations for each correlation: 3 riffles x 7 years) (4 metrics x 11 habitat variables x 6 sites = 264 total correlations). Such analyses produced many correlation coefficients and P-values, with an unknown actual Type I error rate. Thus, a meta-analytic approach was applied in interpreting the results. The number of significant ($P<0.05$) correlations was summarized for each pair of

Table 1. Descriptive statistics, quartiles and scores for aquatic invertebrate metrics calculated using single habitat coarse substrate (riffle) data during a fall index period (from Rabeni *et al*. 1997). Summary statistics are from riffle habitat of reference streams (n=5) in the Ozark ecoregion during the fall index period.

Metric	Statistics				Quartiles			Scores		
	Mean	Standard Error	Minimum	Maximum	25%	50%	75%	5	3	1
Taxa Richness	28.3	3.3	23.5	41.0	21	26	29	>=21	20-11	<11
EPT Richness	13.1	0.7	11.5	15.0	9	11	12	>=9	8-5	<5
HBI	4.3	0.3	3.3	5.0	3.6	4.9	5.3	<=5.3	5.4-7.7	>7.7
Shannon's Diversity Index	2.4	0.1	2.1	2.7	2.29	2.44	2.61	>=2.29	2.28-1.15	<1.15

SCI Scoring: ≥16 not impaired, 10-14 impaired, 4-8 very impaired.

metrics and habitat variables. Then percentage of significant correlations for each pair of metrics and habitat variables, summarized over all metrics, was determined. Although it is unknown which correlations may be spurious, habitat variables with a greater overall percentage of significant correlations are likely to have, in general, greater potential to explain variability in these metrics. SPSS version 20.0 was used to calculate correlation coefficients (IBM Corp. 2011).

Results and Discussion

Aquatic invertebrates

The aquatic invertebrate fauna of the Buffalo River is diverse and many taxa are shared across sampling sites. Among all sites, 167 distinct taxa were identified with similarities ranging from 70% to 83% (Table 2). Because Chironomidae were not identified beyond family level, the number of distinct taxa is likely much higher. Considering the Chironomidae at the family level does not appreciably change the metrics used in this paper (Rabeni and Wang 2001). A complete list of invertebrate taxa at each site, their abundances and associated environmental data are too voluminous to present here, but can be obtained from the senior author. Cluster analysis showed that sites are clustered in a longitudinal progression (Fig. 2). Generally, those sites closest to one another in linear distance were most closely related (Fig. 1, Fig. 2). The exception was site 2, which formed a cluster with site 1 rather than with site 3 as expected, and this cluster was distinct from the remaining sites. This may be partially due to the physical conditions at those sites and stressors acting on the invertebrate communities. Site 1 typically has lower specific conductance (Fig. 8C) and larger substrate size (Fig. 6A) compared to the other sites, and it often has intermittent flows, especially during late summer. Such environmental and habitat conditions are likely reflected in the invertebrate community structure observed at this location. Site 2 is located about 3.5 km downstream of Mill Creek, which has had ongoing high loadings of human coliform bacteria (Usrey 2013). Manner and Mott (1991) found that 96% of the nitrogen load being carried by the Buffalo River below the confluence with Mill Creek was supplied by this stream, and the contamination likely came from the interbasin transfer of groundwater within a nearby watershed.

The metric values recorded clearly exceeded the minimum reference stream values (maximum for HBI) in some years, but not in others (Table 1, Figs. 3A-D). With the exception of HBI, values tended to decrease in a downstream direction to Site 4, and then increase to levels observed upstream. Such variation may not be biologically significant and may be due to the stretch of river upstream of this site experiencing seasonal drying and intermittent flows during most summers. Taxa richness was typically greater than 20 among samples. It is noteworthy that representatives of the intolerant EPT orders were abundant across all sites, and EPT richness values consistently were greater than 12 for all sites in most years. EPT values generally were high relative to Ozark reference streams (Table 1), although not as high as for other regional streams (Bowles *et al*. 2016).

Overall, the invertebrate taxa of the Buffalo River are largely intolerant (mean tolerance value=4.38), and HBI values generally were below 5. Tolerant taxa (tolerance values ≥5) were present in most samples, but they were generally not as well represented in the benthos as intolerant taxa. Individual metrics were highly variable among years and sites, although such among the invertebrate communities shows the importance of using a multi-metric index for stream assessment and multi-year sampling so that too much variability is not unexpected (Mazor *et al*. 2009). HBI values of 5.5 or less are generally considered good, although some organic pollution may be possible (Hilsenhoff 1982, 1988). Mean HBI across years for all

Table 2. Sørensen similarity index for aquatic invertebrate taxa among collecting sites at the Buffalo National River, Arkansas.

	Site 2	Site 3	Site 4	Site 5	Site 6
Site 1	0.70	0.79	0.76	0.76	0.72
Site 2		0.77	0.75	0.73	0.73
Site 3			0.83	0.82	0.80
Site 4				0.81	0.74
Site 5					0.81

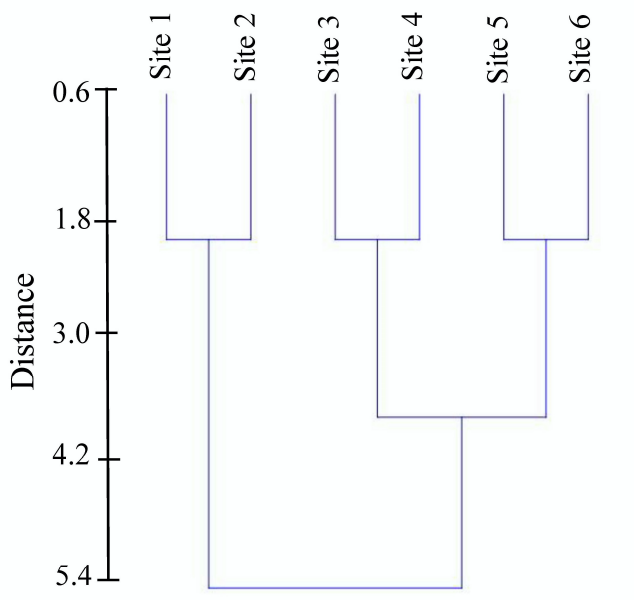
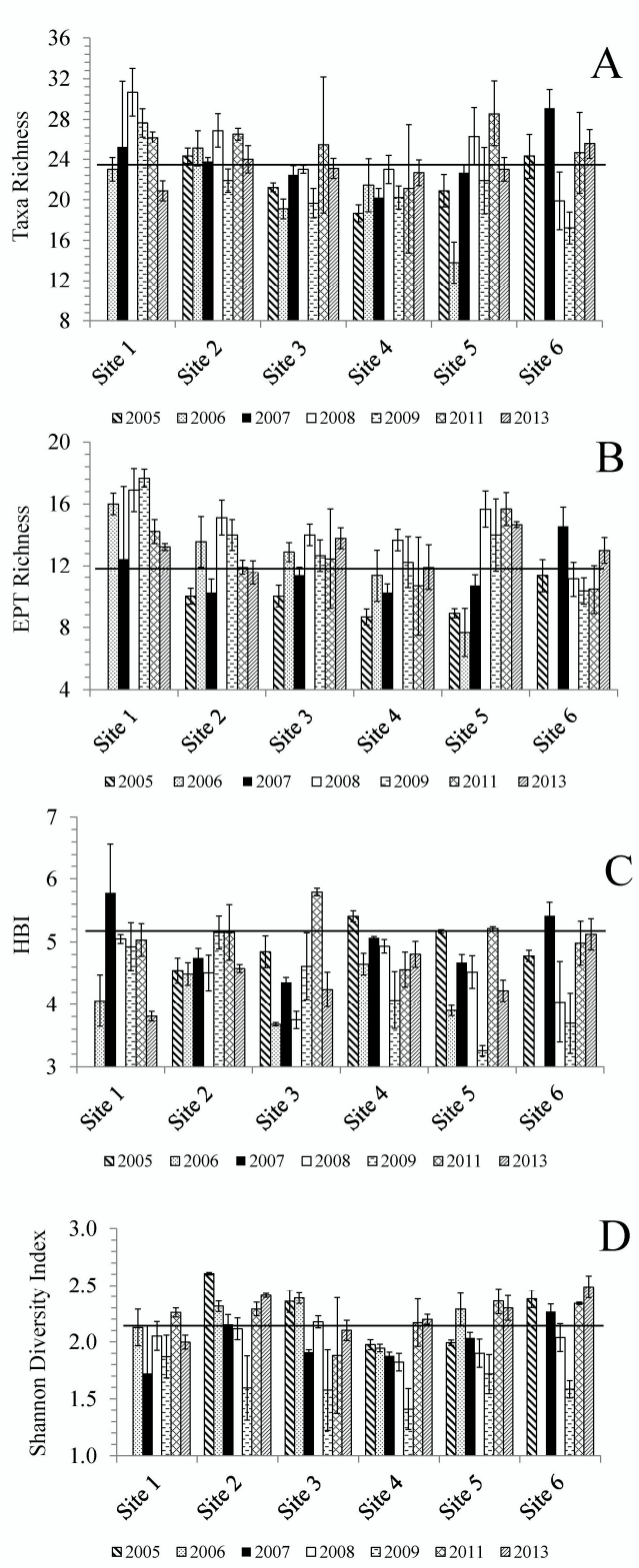


Figure 2. Dendogram showing results for ascendant hierarchical cluster analysis and relative distance of Sørensen's similarity index scores of the aquatic invertebrate communities at sampling sites along the Buffalo River, Arkansas, 2005-2013.

sites ranged from 4.42 to 4.78, which reflects good conditions. Shannon's diversity index values generally ranged from 2.0 to 2.5 among sites and years. Values for Site 4 were generally less than 2, however. For biological data, Shannon's diversity index ranges generally from 1.5 (low species richness and evenness) to 3.5 (high species evenness and richness) (McDonald 2003), but the actual value is contingent on the number of species in the community. The variability observed weight is not placed on the value of a single metric or year. Environmental stressors, such as extended drought and flooding, may impact invertebrate communities and influence assessment results in any given year (Bunn and Arthington 2002; Lake 2003).

SCI scores among sites and years were variable, but they showed that sampling sites are generally not impaired and are fully biologically-supporting (Figs. 4A-F). The lower scores observed in some years are likely due to interannual variability of invertebrate communities coupled with flow dynamics (flood, drought) that occur at those sites rather than anthropogenic disturbances. These data also show the importance of collecting data during multiple years and at multiple sites so that low scores in any given year do not unduly influence management decisions for corrective actions (Mazor *et al*. 2009). SCI scores calculated from data collected during an earlier study conducted by BUFF staff (HTLN 2016) showed a similar response to data collected during this study (site means for all years) with scores being lowest in the mid reaches of the river but then increasing to values similar to those observed upstream (Fig. 5). This finding lends further support to the idea that the losing reaches upstream of site 4 are influencing downstream invertebrate community structure. The higher SCI value for BUFR05 is based on a single sampling event and therefore may not be entirely representative of the range of variation that occurs at that site.

Although the Buffalo River may be classified as relatively high quality, some anthropogenic impacts have occurred there and other threats are ongoing. Previous water-quality and invertebrate community monitoring at BUFF (Mathis 1990; Bryant 1997; Mott 1997; Usrey 2001; Mott and Luraas 2004) showed strong negative correlations between nonpoint source pollution (fecal coliform bacteria, nitrates, phosphorus), stream water quality, and invertebrate community structure along the river's course. In some instances, non-point source pollution has substantial inputs to the river. For instance, Usrey (2001) reported that nitrogen levels of four mid-reach tributaries of the Buffalo River (Mill Creek, Little Buffalo River, Big Creek, and Davis Creek) represented approximately 40% of the total nitrogen loading to the river and average nitrate values were 2 to 4 times higher in these tributaries than in the adjacent river. Usrey (2001) also found that the decreasing abundance of pollution intolerant EPT taxa was associated with higher nitrate concentrations, and increasing orthophosphate concentrations were positively correlated with increasing densities of pollution tolerant dipterans. Inadequately treated wastewater discharges in the Mill Creek watershed continues largely unabated (Watershed Conservation Resource Center 2017). Thus, nutrient loading of the Buffalo River may be among the most significant threats



Figs. 3A-D. Aquatic invertebrate community metrics for the Buffalo River, Arkansas, 2005-2013. Values are means and error bars represent one standard error. The horizontal line conforms to the minimum reported value for Ozark reference streams, except for HBI, which is the maximum reported value (from Rabeni *et al.* 1997).

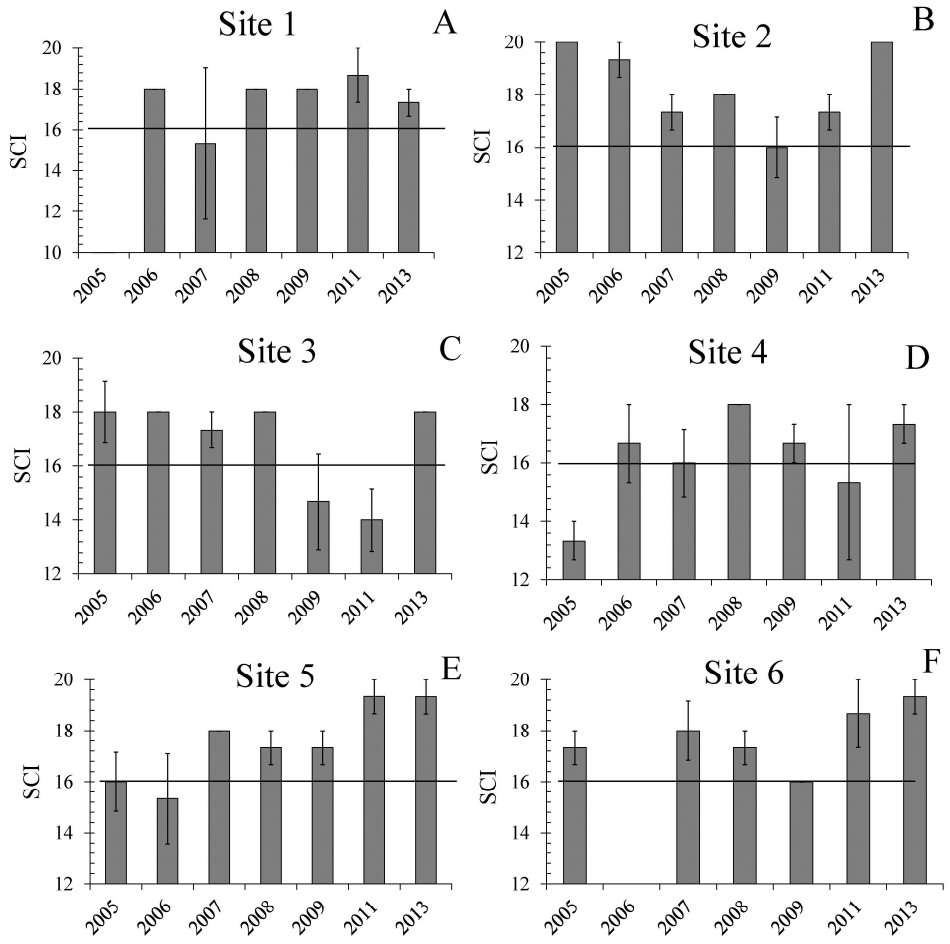
to the integrity of its resident biological communities. The present and previously reported data collectively show the utility of using aquatic invertebrates for assessing water quality integrity. The data also show that mainstem river water quality can be degraded from impairments to tributaries, which in turn degrades biological communities.

Habitat and Water Quality

Mean riffle depth where samples were collected ranged from around 20 to 35 cm, and mean current velocities ranged from about 0.40 to 0.95 m/sec. Substrate was larger at Site 1 (Wentworth Scale=15-16, 45-90 mm, large cobble) compared to the other sites, all of which had similarly sized substrate (Wentworth Scale=13-15, 32-64 mm, large pebble) (Fig. 6A). Substrate embeddedness was similar at most sites generally, ranging from 25 to 30%, but was least at the upstream most site (~20%) and slightly higher at the downstream most site (~40%) (Fig. 6B).

Aquatic vegetation (mostly mosses) and filamentous green algae were poorly represented at all sampling sites (<20%) and those data are not presented here. Periphyton densities growing on the rock substrates were generally consistent at the upper 3 sampling sites and at site 6 (~25%), but were frequently higher at sites 4 and 5 (~35% and 30%, respectively) (Fig. 7). Sites 4 and 5 are downstream of the Woolum Access of the Buffalo River and this stretch of river has two prominent losing reaches where surface flows are periodically diverted completely to subsurface flow, especially during summer (Moix and Galloway 2004). These losing reaches are approximately 5 km and 4.5 km long, respectively, and are separated by a 4 km long gaining reach. The latter losing reach ends less than 1 km upstream of site 4. It is possible that this losing reach located above the sampling site may stimulate increased growth of periphyton at those sites due to increased temperatures and nutrient loading associated with the resulting pooling of the river (Petersen and Femmer 2002). Upstream nutrient loading from tributaries could also play a role in stimulating growth. Shorter losing reaches (~2 km) are located in the upper Buffalo River including one located immediately upstream and partially overlapping with site 1, but that site has been dewatered only once during our sampling window (2005).

Habitat conditions were generally consistent among sites and years. Overall, no habitat variables exhibited persistently strong correlations with any of the metrics, and the percentage of "significant" correlations was relatively low (<30%) in all cases (Table 4). In addition,



Figs. 4A-F. Mean SCI values and standard errors for collecting sites on the Buffalo River, 2005-2013. The horizontal line represents an SCI of 16, the lower limit for rating a site unimpaired.

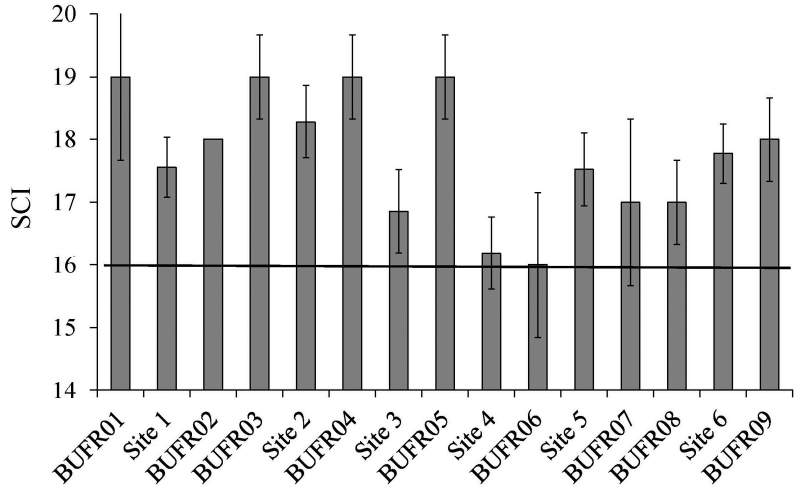


Figure 5. Mean SCI scores and standard errors for Buffalo River water quality bioassessment sampling sites collected in 2005 and Heartland Inventory and Monitoring Network sampling sites (2005-2013). See Figure 1 for site locations. The horizontal line represents an SCI of 16, the lower limit for rating a site unimpaired.

Table 3. Summary of BUFF pairwise correlations (Kendall's tau) organized by year (i.e., correlations conducted among all sites in each year) and by site (i.e., correlations conducted among all years at each site). Values are number of significant correlations/percentage of significant correlations of total.

Variables	HBI	Taxa Richness	EPT Richness	Shannon's Diversity	**Total**
		By Year			
Filamentous algae	2/0.29	2/0.29	1/0.14	1/0.14	6/0.21
Current velocity	1/0.14	2/0.29	1/0.14	1/0.14	5/0.18
Dissolved oxygen	2/0.29	1/0.14	1/0.14	1/0.14	5/0.18
Temperature	2/0.29	1/0.14	0/0	1/0.14	4/0.14
Substrate size	1/0.14	1/0.14	2/0.29	0/0	4/0.14
Specific conductance	2/0.29	0/0	1/0.14	1/0.14	4/0.14
Substrate embeddedness	0/0	1/0.14	2/0.29	0/0	3/0.11
pH	0/0	1/0.14	1/0.14	1/0.14	3/0.11
Periphyton	1/0.14	1/0.14	1/0.14	0/0	3/0.11
Depth	0/0	1/0.14	1/0.14	0/0	2/0.07
Vegetation	1/0.14	0/0	0/0	0/0	1/0.04
Total	12/0.16	11/0.14	11/0.14	6/0.08	40/0.13
	Expected number of spurious correlations = 15				
		By Site			
Filamentous algae	0/0	3/0.50	4/0.67	0/0	7/0.29
Current velocity	1/0.17	2/0.33	2/0.33	1/0.17	6/0.25
Dissolved oxygen	2/0.33	1/0.17	1/0.17	2/0.33	6/0.25
Temperature	0/0	2/0.33	2/0.33	1/0.17	5/0.21
Substrate size	2/0.33	1/0.17	0/0	1/0.17	4/0.17
Specific conductance	1/0.17	1/0.17	1/0.17	1/0.17	4/0.17
Substrate embeddedness	0/0	1/0.17	2/0.33	1/0.17	4/0.17
pH	1/0.17	0/0	1/0.17	1/0.17	3/0.13
Periphyton	1/0.17	0/0	1/0.17	1/0.17	3/0.13
Depth	0/0	0/0	0/0	1/0.17	0/04
Vegetation	0/0	0/0	0/0	0/0	0/0
Total	8/0.12	11/0.17	14/0.21	10/0.15	43/0.16
	Expected number of spurious correlations = 13				

a certain number of spurious correlations are expected (1 in 20 for alpha = 0.05) in analyses such as those conducted here. The number of expected spurious correlations ranged from 22 to 38% of the observed "significant" correlations (Table 3). Algae, current velocity, dissolved oxygen, temperature, substrate, and specific conductance usually had a greater percentage of "significant" correlations than the other variables, across all analyses, but some of these variables are autocorrelated, hence their biological significance may not be relevant (Martínez-Abraín 2008). The low number of significant correlations for some habitat variables is likely due to the categorical scale used to assess some habitat data (see Methods), and the lack of variability in the values observed for these variables. This analysis shows that the habitat data collected in relation to benthic invertebrate samples presently has limited value for correlating with community and diversity metrics, but that finding does not rule out further analyses with individual invertebrate taxa or groups of

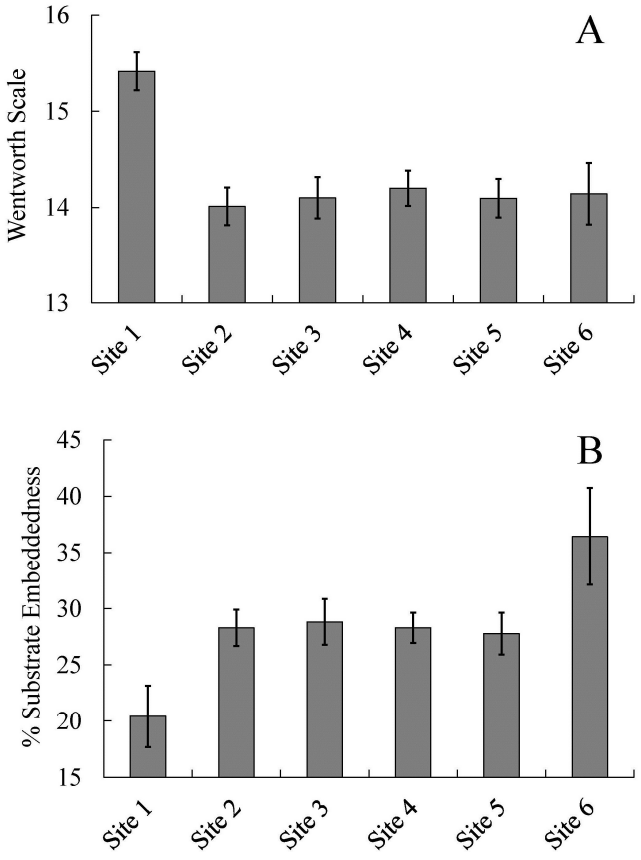


Figure 6A-B. Mean substrate size (Wentworth scale) and percent substrate embeddedness associated with benthic invertebrate samples from the Buffalo River, Arkansas, 2005-2013. Error bars represent one standard error.

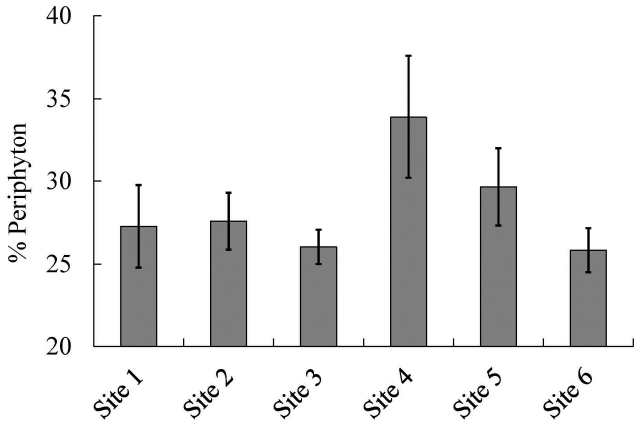


Figure 7. Percent periphyton associated with benthic invertebrate samples from the Buffalo River, Arkansas, 2005-1013. Values are means; error bars represent one standard error.

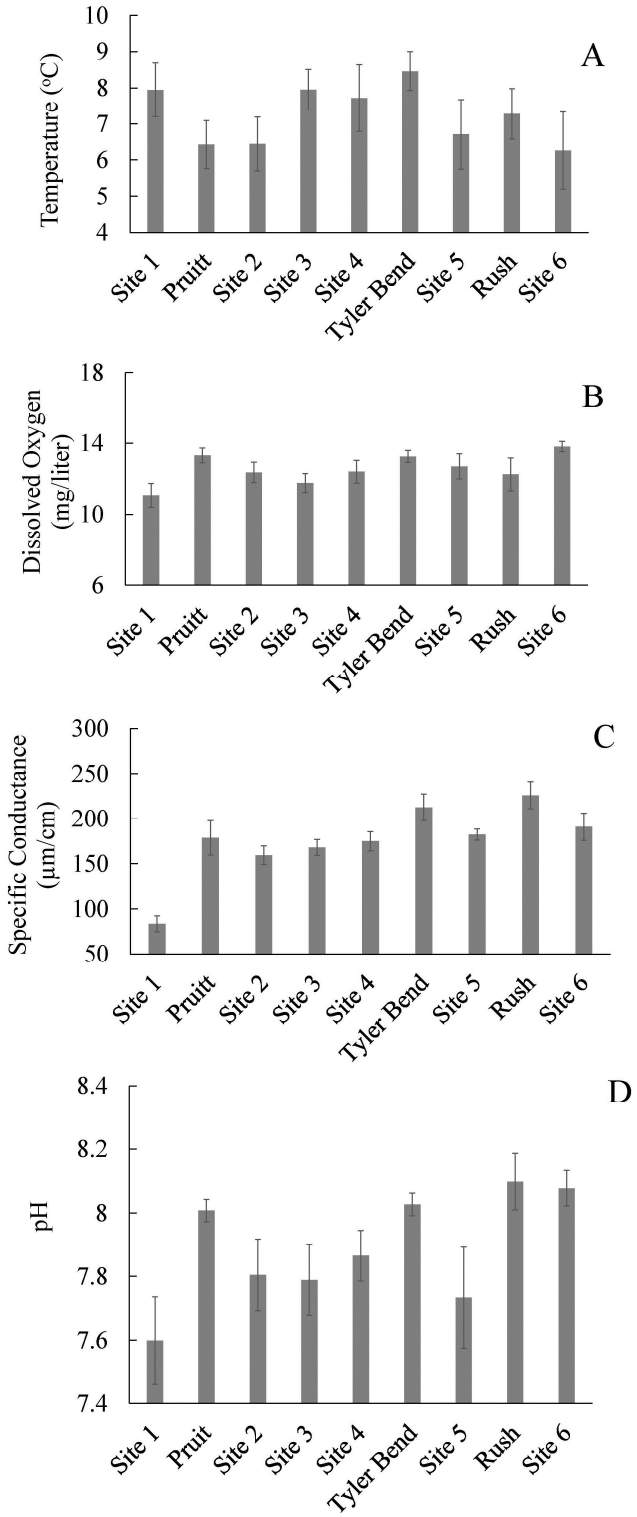


Figure 8A-D. Water physical-chemical data for sampling sites on the Buffalo River, Arkansas, 2005-2013. Values are means with standard errors. Data were collected as static readings using hand-held meters at sampling sites 1-6, while data were collected continuously using dataloggers at Pruitt, Tyler Bend and Rush locations. See methods for other details.

taxa (e.g., EPT). Accordingly, only summary data are presented here to generally characterize the conditions in which samples were collected, and a further analysis of this data is beyond the scope of this paper.

Water quality met state standards in all instances (Arkansas Pollution Control and Ecology Commission 2017) (Fig. 8A-D). Temperature was variable among sampling sites and years, which was expected due to climatic variations among years sampled as well as location of sampling sites along the length of the river. Dissolved oxygen levels were high in all instances and were at or above saturation across years and sites (means=11.1-13.9, range 8.4-15.3 mg/liter). Specific conductance was lowest at the upstream most sampling site across years (mean=83.5 µm/cm, range 48.5-126.7 µm/cm) while mean values increased in a downstream direction for the other sites (means=154, 170, 175, 184 and 192, respectively µm/cm). pH was consistent and similar among all sampling sites and years sampled (means=7.6-8.1), and values are reflective of the karst topography of the Buffalo River basin. Turbidity, not shown here, was nearly always below 10 NTU. The water quality values we report are consistent with those recorded by other studies (Moix and Galloway 2004, Huggins *et al*. 2005, Watershed Conservation Resource Center 2017) with the exception of temperature because their data were recorded during different seasons.

Conclusions

This paper provides baseline invertebrate, habitat and water quality data for selected sites on the Buffalo River, Arkansas. Invertebrate community structure in the Buffalo River generally is diverse and reflects above average water quality. Inherent variability of invertebrate community diversity and density across sites and years highlights the importance of multiyear assessment and monitoring to support management decisions. Although the condition of invertebrate communities and water quality in the Buffalo River exceeded water quality standards and have high integrity, numerous ongoing and projected threats to these resources remain, and those threats largely originate outside of the park's jurisdictional boundaries. Aquatic invertebrate monitoring at BUFF provides a sound tool to recognize both deterioration and chronic decline of water quality.

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Bat Occupancy Estimates and Species Richness at Cache River National Wildlife Refuge

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Running title: Occupancy Estimates of Bats of Cache River National Wildlife Refuge

Abstract

Six bat species of special concern, threatened or endangered, may occur in one of Arkansas' largest bottomland hardwood forests, the Cache River National Wildlife Refuge (CRNWR). However, inventory of bat species throughout the refuge has been lacking and management plans may not be adequate in promoting bat conservation. The objectives of this study were to inventory bat species in the CRNWR, and determine bat-habitat associations via occupancy estimates. From May–August 2014 and 2015, we mist-netted from sunset for 5 hours. We also deployed bioacoustic devices throughout 5 habitat types (cypress-tupelo [dominantly *Taxodium distichum* and *Nyssa aquatica*], emergent wetland, mature forest, hardwood reforestation, and managed hardwood). Mist-netting yielded 460 bat captures with Rafinesque's big-eared bats (*Corynorhinus rafinesquii; n* = 156) being the most common capture, followed by eastern red bats (*Lasiurus borealis*; *n* = 104), southeastern myotis (*Myotis austroriparius; n* = 91), evening bats (*Nycticeius humeralis*; *n* = 58), tri-colored bats (*Perimyotis subflavus*; *n* = 54), and a big-brown bat (*Eptesicus fuscus*; *n* = 1). Based on 3,896 calls identified with 85% certainty, evening bats and rarer big-brown bats tended to occupy managed hardwood forests more than any other habitat (occupancy probabilities ± SE: $\Psi = 0.75 \pm 0.13$ and 0.38 ± 0.19, respectively). Tri-colored bats tended to be more present in mature forest habitats ($\Psi = 0.91 \pm 0.09$), and *Myotis* species tended to have highest occupancy rates in cypress-tupelo stands ($\Psi = 0.59 \pm 0.15$). Not all species were detected with both methods. Thus, we encourage future studies to combine mist-netting and acoustic surveying methods to minimize bias in species presence estimate. This would ensure management practices that would benefit all present species.

Introduction

Since the colonization by European settlers, America's bottomlands have been greatly reduced and converted for agricultural use (Dahl 1980; Hank and Gosselink 1990). Only 10% of the original wetland habitat in the Mississippi Alluvial Plain remains today (Stanturf *et al*. 2000). The Cache River National Wildlife Refuge (CRNWR), when combined with other nearby conservation holdings, forms the second largest contiguous tract of forested wetland in Arkansas. The 27,315-ha refuge, founded in 1986 and located within Jackson, Woodruff, Monroe, and Prairie counties, is composed of bottomland hardwood forests (19,592 ha), reforested land (6,282 ha), and cropland and moist-soil units (1,441 ha). The CRNWR also borders several state wildlife management areas (WMA) such as Sheffield Nelson Dagmar WMA and Rex Hancock Black Swamp WMA as well as land owned by Arkansas Natural Heritage Commission. The CRNWR is listed on The Ramsar Convention of Wetlands as one of the Wetlands of International Importance in the United States, with 510 species of fauna and 120 species of trees and shrubs within the refuge (The Annotated Ramsar List: United States of America 2013).

Arkansas supports 16 bat species, 10 of which occur in the same counties as the CRNWR (Sealander and Heidt 1990). Two of these 10 species have some level of federal protection: the Indiana bat (*Myotis sodalis*), is listed as federally endangered, and the northern long-eared bat (*M. septentrionalis*) is threatened. Additionally, in Arkansas, the Rafinesque's big-eared bat (*C. rafinesquii*) and southeastern myotis (*M. austroriparius*) are species of special concern, and the little brown bat (*M. lucifugus*) is listed as a species of greatest conservation concern.

Several studies have examined the distribution of bats in bottomland forests of Arkansas (Fokidis *et al.*

2005; Medlin 2006; Medlin *et al*. 2006). However, no study has exclusively focused on the bats of the CRNWR. Our first objective was to inventory bat species of the CRNWR. Our second objective was to estimate bat occupancy (i.e., the probability that a site selected at random is occupied by a species) in different habitats within the refuge. We hypothesized that habitat usage of the southeastern myotis and Rafinesque's big-eared bat is similar because both species are known to roost in cypress-tupelo dominated habitat (Jones and Manning 1989; Rice 1957, 2009; Stuemke *et al*. 2014). Therefore, we predicted that occupancy of *Myotis* bats and Rafinesque's big-eared bat would be highest in cypress-tupelo habitat compared to other habitats. Additionally, we hypothesized that habitat usage for other bat species is more flexible because of their wider distribution. Therefore, occupancy among species should be similar and reflect availability of other habitat types (Sealander and Heidt 1990; Fokidis *et al*. 2005; Medlin *et al*. 2006). The findings and conclusions in this article are those of the author(s) and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

Materials and Methods

We captured bats over 45 nights during May 15 – August 15, 2014 and 2015 using 3–4 triple-high, 38-mm meshed mist-nets (AviNet Inc., New York, USA) of varying lengths. We sampled 21 netting locations, 1-3 times each, and spread our netting effort across the reproductive season. We chose netting locations in corridors with enclosed low canopies (e.g., roadways, waterways) to funnel bats into nets. We opened nets at sunset for 5 hours and checked for bat captures every 10 min, following U.S. Fish and Wildlife Service (USFWS) Indiana Bat protocol (USFWS 2016). All capture and handling procedures followed the guidelines of the American Society of Mammalogists for animal use (Sikes *et al*. 2011) and were approved by the Arkansas State University Institutional Animal Care and Use Committee (protocol 451729-1).

We used 2 bioacoustics approaches. In approach A, AnaBat SD2 Active Bat Detectors (Titley Electronics, Columbia MO) complemented netting efforts in both years. We deployed SD2 units, placed in modified ammunition boxes, before sunset on a 1-m tall PVC pipe anchored to the ground within 75 m of net-sites in fields, corridors or the interior of the forest. We collected detectors while nets were being closed for the night. Additionally, in approach B, from May–August, 2015, we collected search-phase echolocations of bats using 5 AnaBat SD2 units in 5 pre-defined habitat types: cypress-tupelo (dominated by *Taxodium distichum* and *Nyssa aquatica*; covering 7% of the CRNWR), reforestation (most trees were 10-20 years old; 21% coverage), mature forest (i.e., extant forest never cleared for agriculture; 65% coverage), managed hardwood (received some sort of silvicultural treatment; 4% coverage), and emergent wetland (moist-soil units, agricultural or open fields; 3% coverage). Each detector recorded calls for 3-5 consecutive nights in 16-20 stands (replicates) for each of the 5 habitat types, for a total of 91 sites. We programmed detectors to sample 30 min before sunset until 30 min after sunrise.

We classified search-phase echolocation calls of bats to species using Bat Call Identification version 2.7c (BCID, Kansas City, Missouri). We included only bats species whose range overlaps with the CRNWR in the analysis, i.e., eastern red bat (*Lasiurus borealis*), tri-colored bat (*Perimyotis subflavus*), Rafinesque's big-eared bat, big brown bat (*Eptesicus fuscus*), hoary bat (*L. cinereus*), evening bat (*Nycticeius humeralis*), and *Myotis* bats. The distribution of Brazilian free-tailed bats (*Tadarida brasiliensis*) only overlaps the southernmost portion of Prairie County and was therefore not included in the analysis. Although the Seminole bat's (*L. seminolus*) range overlaps with the CRNWR, reference libraries were not available in BCID or in EchoClass. We restricted calls to those containing at least 5 pulses (Mora *et al.* 2011) and we only retained those with a probability of correct species identification of $\geq$0.85. We then visually vetted retained calls with Analook 4.1 (Titley Electronics, Columbia, Missouri) to ensure accuracy. However, due to similarities in call structure between the Indiana bat, southeastern myotis, little brown bat, and northern long-eared bat, we placed all *Myotis* calls into one category.

We used only acoustic data collected under approach B to estimate single-season occupancy and probability of detection for each species in Program PRESENCE version 10.5 (US Geological Survey, Laurel, Maryland). Single-season occupancy models have 3 assumptions that must be met (MacKenzie *et al*. 2002): (1) sites are closed to changes in occupancy, which we met by having short sampling periods during which changes in occupancy are least likely to occur through volancy, death, or recruitment; (2) species are never falsely detected when absent, which we attempted to address by visually vetting calls; and (3) detection of a species at a site is independent of detecting the species at all other sites, which we met by having a single acoustic detector in each habitat type during each survey. We visually vetted calls by split-screen

comparisons of collected and known calls, and by using an acoustic guidebook provided by Titley Electronics (Columbia, Missouri). Occupancy models estimate the probability of detection (*p*), i.e., the proportion of animals present that are detected, and the occupancy (Ψ) corrected by *p*, i.e., the probability that a site selected at random or sampling unit in a single area is occupied by a species (MacKenzie *et al*. 2006). For each species group, we conducted our analyses in three steps. First, we compared models with constant and survey-specific *p* while keeping Ψ constant. Models with constant *p* assign each survey effort the same probability of detection and estimate the highest probability of detection, whereas survey-specific *p* models assign probabilities of detection for each night of each survey effort. Second, starting with the best general structure for *p*, we compared *p* models with covariates (Julian date for a possible temporal trend and habitat types), keeping Ψ constant. Finally, using the best *p* model, we selected the best Ψ model with Julian date and habitat type as possible covariates. For all comparisons, an Akaike Information Criterion (AIC) was used to select the best model, i.e., the model with the lowest AIC (Burnham and Anderson 2002).

Results

Mist-netting yielded 460 bat captures for 45 trap-nights and 21 sites. The most common capture was the Rafinesque's big-eared bat (*n* = 156; Fig. 1), followed by eastern red bat (*n* = 104), southeastern myotis (*n* = 91), evening bat (*n* = 58), tri-colored bat (*n* = 54), and a Prairie County record for big brown bat (*n* = 1).

Table 1 – Bat occupancy model selection with constant (*p.*) versus survey-specific (p_s) detection probability. AIC, ΔAIC, and AICwt are Akaike Information Criterion, the difference in AIC for each pair of models, and the relative support of the model, respectively. The estimate of constant detection probability is indicated with its standard error.

Models	AIC	ΔAIC	AICwt	*p* (± SE)
Myotis bat				
p.	196.16	0.00	0.970	0.344 ± 0.071
p_s	203.12	6.96	0.030	
Tri-colored bat				
p.	324.64	0.00	0.755	0.833 ± 0.023
p_s	330.04	2.25	0.245	
Eastern red bat				
p.	99.19	0.00	0.959	0.073 ± 0.068
p_s	105.50	6.31	0.041	
Evening bat				
p.	347.17	0.00	0.881	0.423 ± 0.047
p_s	354.12	4.01	0.119	
Big brown bat				
p.	161.43	0.00	0.778	0.225 ± 0.074
p_s	163.94	2.51	0.222	

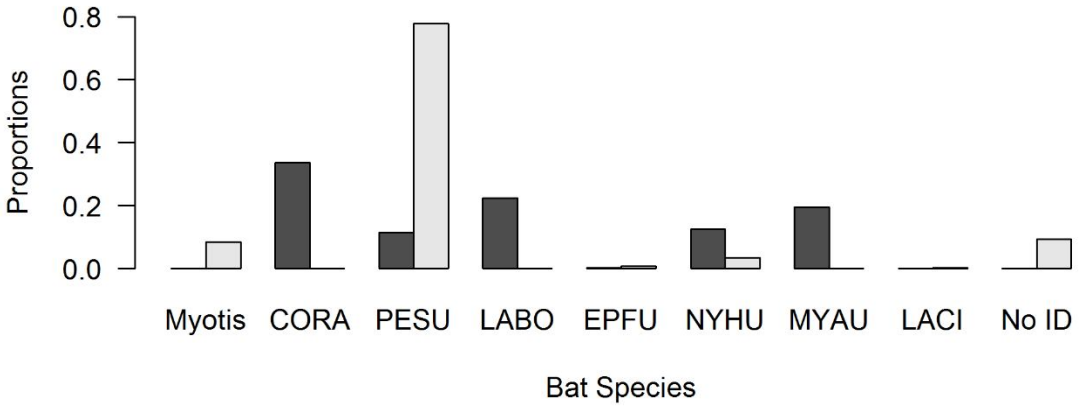

Figure 1. Proportions of individual bats captured through mist- netting (black) and call files collected through bioacoustics (gray), in 2014–2015, for Rafinesque's big-eared bat (CORA), eastern red bat (LABO), southeastern myotis (MYAU), *Myotis* species, evening bats (NYHU), tri-colored bat (PESU), big-brown bat (EPFU), hoary bat (LACI), and bat calls not identified to species (No ID). *Myotis* species were pooled together for the bioacoustics count because these species have similar calls and could not be distinguished with certainty. Bioacoustic data collected with two approaches (i.e., by net sites both years, and in 5 pre-defined habitat types in 2015 only) were pooled.

Acoustic data collected with both approaches totaled 4,640 call files identified to species (approach A: n_A = 744 calls; approach B: n_B = 3,896 calls) and 483 call files recognized as bats but not identified to species. By decreasing order, 4,010 files (n_B = 3,434) were identified as tri-colored bats (Fig. 1), 426 (n_B = 277) as *Myotis* bats, 180 (n_B = 166) as evening bats, 39 (n_B = 34) as big-brown bats, 9 (n_B = 9) as hoary bats, 4 (n_B = 3) as eastern red bats, and 2 as Rafinesque's big-eared bats (n_B = 0).

Occupancy models with a constant (as opposed to survey-specific) probability of detection were the best models for all species and habitat types (Table 1). Probability of detection depended on habitat types for *Myotis* species and tri-colored bats (Table 2). *Myotis* were significantly less detected in managed forest than in cypress-tupelo stands, whereas tricolored bats were significantly more detected in managed hardwood than in mature forest (Table 3). On the contrary, detectability was constant for evening, big brown, and eastern red bats (Table 2).

The overall occupancy was (constant model) was highest for tri-colored bats (0.840 ± 0.039 [SE]), followed by evening bats (0.599 ± 0.069) and *Myotis* (0.301 ± 0.067). Big brown bats' occupancy (0.319 ± 0.101) (0.319 ± 0.101) did not differ from *Myotis* or evening bats, and the estimate for eastern red bats (0.475 ± 0.425) was associated with large uncertainty (Fig. 2). Constant occupancy was the most supported model for evening, tri-colored, and big brown bats, indicating no habitat was significantly more occupied by any of these species (Tables 3 & 4). However, tri-colored bats tended to occupy mature forest slightly more and evening bats had relatively higher occupancy in managed hardwood forests (Table 3). Occupancy for *Myotis* increased with time ($slope_{JD}$ = 0.019 ± 0.003), but did not vary among habitat types although they tended to be more present in cypress-tupelo habitats (Table 3).

For eastern red bats, the best model indicates that occupancy varied among habitat types (Table 4), but it could not be estimated for two habitat types and the uncertainty for the estimated occupancy in the other three types was large (Table 3). Rafinesque's big-eared bats had too few confirmed calls to run occupancy analysis.

Table 2 – Model selection for bat detection probability modeled as constant or as a function of habitat types and Julian date. Occupancy was kept constant. AIC, ΔAIC, and AICwt are Akaike Information Criterion, the difference in AIC for each pair of models, and the relative support of the model, respectively.

Models	AIC	ΔAIC	AICwt
Myotis			
Habitat	193.28	0.00	0.553
Habitat + Julian date	195.25	1.97	0.206
Constant	196.16	2.88	0.131
Julian date	196.51	3.23	0.110
Tri-colored bat			
Habitat	321.72	0.00	0.591
Habitat + Julian date	323.69	1.97	0.221
Constant	324.64	2.92	0.137
Julian date	326.61	4.89	0.051
Eastern red bat			
Constant	99.19	0.00	0.425
H	99.85	0.66	0.306
Julian date	101.19	2.00	0.156
Habitat + Julian date	101.85	2.66	0.112
Evening bat			
Constant	347.17	0.00	0.667
Julian date	348.76	1.59	0.301
Habitat	353.91	6.74	0.023
Habitat + Julian date	355.69	8.52	0.009
Big brown bat			
Constant	161.43	0.00	0.701
Julian date	163.41	1.98	0.261
Habitat	167.89	6.46	0.028
Habitat + Julian date	169.88	8.45	0.010

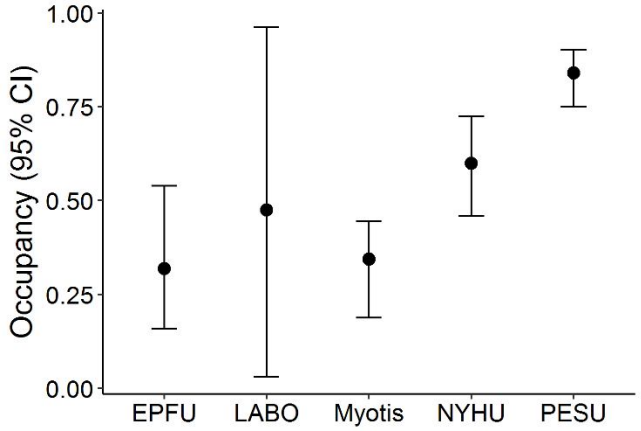

Figure 2 - Overall occupancy using constant model for eastern red bat (LABO), southeastern myotis (MYAU), *Myotis* species, evening bats (NYHU), tri-colored bat (PESU), and big-brown bat (EPFU) with 95% confidence intervals.

Discussion

The CRNWR's bat community included 6 species detected via acoustics that were also physically confirmed via capture in mist-nets. One other species,

Table 3 – Occupancy estimates (Ψ) and detection probabilities (*p*) for *Myotis* species, tri-colored, eastern red, evening, and big-brown bats in each pre-defined habitats (i.e., CT = cypress-tupelo; EW = emergent wetland, MF = mature forest, RF = reforestation, and MH = managed hardwood) of the Cache River National Wildlife Refuge for 2015. All *p* estimates are from models with Ψ constant and habitat-dependent *p*, whereas Ψ estimates are from models with habitat-dependent Ψ and *p* from the best species-specific model in Table 2.

Habitat	Ψ (95% CI)	*p* (95% CI)
Myotis bat		
CT	0.593 (0.296 – 0.835)	0.453 (0.284 – 0.634)
EW	0.174 (0.296 – 0.436)	0.255 (0.048 – 0.700)
MF	Not estimable	0.042 (0.005 – 0.273)
RF	0.229 (0.083 – 0.496)	0.341 (0.091 – 0.729)
MH	Not estimable	0.141 (0.043 – 0.375)
Tri-colored bat		
CT	0.834 (0.591 – 0.946)	0.845 (0.719 – 0.921)
EW	0.896 (0.661 – 0.975)	0.883 (0.773 – 0.943)
MF	0.909 (0.548 – 0.988)	0.674 (0.529 – 0.792)
RF	0.753 (0.522 – 0.894)	0.835 (0.699 – 0.917)
MH	0.843 (0.608 – 0.949)	0.909 (0.798 – 0.962)
Eastern red bat		
CT	Not estimable	0.092 (0.013 – 0.443)
EW	0.602 (0.070 – 0.968)	0.055 (0.007 – 0.341)
MF	Not estimable	Not estimable
RF	0.200 (0.016 – 0.793)	0.018 (0.001 – 0.212)
MH	0.210 (0.017 – 0.808)	0.037 (0.004 – 0.289)
Evening bat		
CT	0.588 (0.314 – 0.817)	0.488 (0.305 – 0.675)
EW	0.623 (0.343 – 0.840)	0.352 (0.206 – 0.533)
MF	0.454 (0.205 – 0.729)	0.407 (0.214 – 0.634)
RF	0.545 (0.288 – 0.781)	0.459 (0.269 – 0.662)
MH	0.750 (0.426 – 0.923)	0.419 (0.272 – 0.583)
Big brown bat		
CT	0.279 (0.076 – 0.646)	0.202 (0.049 – 0.556)
EW	0.363 (0.109 – 0.727)	0.287 (0.092 – 0.613)
MF	0.210 (0.045 – 0.600)	0.107 (0.019 – 0.424)
RF	0.362 (0.106 – 0.731)	0.257 (0.089 – 0.552)
MH	0.377 (0.111 – 0.747)	0.195 (0.067 – 0.450)

Table 4 – Model selection for bat occupancy modeled as constant or as a function of habitat types and Julian date. Detection probabilities were constant for eastern red, evening, and big brown bats, but were modeled as a function of habitat types for Myotis and tri-colored bats. AIC, ΔAIC, and AICwt are Akaike Information Criterion, the difference in AIC for each pair of models, and the relative support of the model, respectively.

Models	AIC	ΔAIC	AICwt
Myotis			
Julian date	192.31	0.00	0.388
Constant	193.28	0.97	0.239
Habitat + Julian date	193.67	1.36	0.196
Habitat	193.88	1.57	0.177
Tri-colored bat			
Constant	321.72	0.00	0.953
Habitat	327.75	6.03	0.047
Julian date	394.06	72.34	0.000
Habitat + Julian date	402.06	80.34	0.000
Eastern red bat			
Habitat	97.97	0.00	0.408
Habitat + Julian date	98.66	0.69	0.289
Constant	99.19	1.22	0.222
Julian date	101.19	3.22	0.082
Evening bat			
Constant	347.17	0.00	0.678
Julian date	349.04	1.87	0.266
Habitat	352.77	5.60	0.041
Habitat + Julian date	354.75	7.58	0.015
Big brown bat			
Constant	161.43	0.00	0.621
Julian date	162.57	1.14	0.351
Habitat	168.58	7.15	0.017
Habitat + Julian date	169.52	8.09	0.011

the hoary bat, was detected acoustically only. The presence of 4 of these 6 species (eastern red, big brown, evening, and tri-colored bats) was not surprising, as they are fairly common throughout the state (Fokidis *et al*. 2005; Sealander and Heidt 1990). However, proportions of captures and calls were not in agreement (Fig. 1). Only two calls were recorded for the most commonly captured species, the Rafinesque's big-eared bat, whereas the most common species acoustically, the tri-colored bat, was the second least common capture. Rafinesque's big-eared bats may have been under-detected due to the ineffectiveness of zero-cross systems such as AnaBat systems (Hein *et al*. 2009) and because bats within the genus *Corynorhinus* echolocate at low intensities that are hard to detect, hence their nickname of "whispering bats" (Fenton 1982; Lacki and Bayless 2013; Loeb *et al*. 2015; Stihler 2011). Additionally, the tri-colored bat was the second least common capture via mist-netting but dominated acoustic surveys accounting for 81% of all identified call files. These findings are

similar to other acoustic studies that reported low numbers of captures but high numbers of confirmed calls for this species (Young and Gruver 2011; Jordan 2014). The probability of detection for tri-colored bats was higher than for all other species. The amplitude of the species' echolocation is higher than in other species, which may inflate their detectability by zero-cross devices (Ryan Allen, *pers. comm.*; MacDonald *et al.* 1994), but may not necessarily reflect their relative abundance. Such inflated detection has the potential to bias conclusions. Also, although tri-colored bat calls are rather unique, we cannot rule out the possibility of some calls of other species being misclassified as tricolored bats. Although habitat type affected detection probabilities, the tri-colored bat was seemingly a generalist, not preferring any one habitat. They had a tendency to be more present in mature forest, as expected based on availability since mature forest represented the main habitat type (65%) in the refuge.

The loudness of tri-colored bats and quietness of Rafinesque's big-eared bat may lead to overestimated and underestimated occupancy estimates, respectively. Furthermore, the eastern red bat had the second highest physical capture rate among our 6 species, but it was also among the least common identified bat calls, despite higher frequencies than Rafinesque's big-eared bat. Eastern red bat calls may have been misclassified as evening bats (Britzke 2003), and these two species may need to be considered as one LABO/NYHU group in future studies (Cox *et al*. 2016).

As expected, *Myotis* bats tended to have higher occupancy at cypress-tupelo stands more than any other habitat type even though cypress-tupelo stands comprised 7% of the refuge. Although *Myotis* bats were placed into one category due to similarities of call structure, the *Myotis* bats' affinity toward cypress-tupelo stands could be reflective of the strong associations with bottomland hardwood forests of the southeastern myotis, the only Myotine bat captured during the study (Gooding and Langford 2004; Jones and Manning 1989; Rice 1957; Stuemke *et al*. 2014). Thus, the higher occupancy in cypress-tupelo habitats may suggest dominance of southeastern myotis over other *Myotis* bats. Due to similarities of *Myotis* calls, presence of the northern long-eared bat and Indiana bat on the CRNWR should not be excluded. An Indiana bat had possibly been detected acoustically in Jackson County in the summer of 2013 (Richard Crossett, *pers. observ.*). Capture at emergence and radio-tracking may provide more data to inform us about the likelihood of these species using the Delta in general and CRNWR specifically. Finally, we were not able to test the prediction of a higher occupancy of Rafinesque's big-eared bats in cypress-tupelo habitats because of a lack of acoustic data, but we confirmed its presence in the refuge.

Acoustic data were in agreement with mist-netting data for big brown bats, both suggesting its rarity within the refuge. Although studies in highlands of the Ouachita Mountains (Saugey *et al*. 1989) and in the southeastern portion of the state where bottomland forests are present (Baker and Ward 1967) showed low capture rates of both Rafinesque's big-eared bats and southeastern myotis, higher captures of both species were reported in the east-central portion of the state (Fokidis *et al* 2005; Medlin *et al.* 2006). Higher numbers in the CRNWR may relate to overall suitability of the refuge for these two species. The CRNWR is also on the westward edge of their distribution (Arroyo-Cabrales and Álvarez-Castañeda 2008a,b) and the Arkansas Delta represents their core population areas in the state (Fokidis *et al*. 2005; Medlin *et al*. 2006). Big brown bats as well as evening bats tended to have higher occupancies in managed hardwood forest, which suggests that these two species may share a preference for habitats with a more open canopy as a result of silviculture treatments (Timpone *et al.* 2006; Istvanko *et al*. 2016). Therefore, our results only partially supported our prediction that habitat use would reflect habitat availability.

Conclusions

The results of this study show the value of a two-pronged method to surveying bats. Passive surveying methods such as bioacoustics can complement physical methods. Despite the similar echolocation calls among *Myotis* species and the current inability of bioacoustic devices to detect low-frequency calls of big-eared bats, acoustic monitoring is becoming a more standard and cheaper approach to bat research and can be used to assess spatiotemporal patterns of bat activity. Similarly, mist-netting provides physical evidence of a species presence although high-fliers such as hoary bats are more likely to be missed (Brown 1997). Therefore, it is recommended to combine acoustic surveys with mist-netting to confirm species presence or absence (Kaiser and O'Keefe 2015). If land managers of the CRNWR based management decisions solely off acoustic data, these decisions would not necessarily promote the most common bat species (i.e., Rafinesque's big-eared bat). In addition, due to zero-cross systems' seemingly ineffective ability at detecting low-amplitude bat calls, land managers could consider using full-spectrum

detectors to increase their chances of detecting the "whispering bats". Finally, this study provides land managers with a weighted guideline of how management practices in certain habitat types may affect bat species and can provide guidance during their decision making process.

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Leech Parasitism of the Gulf Coast Box Turtle, *Terrapene carolina major* (Testudines:Emydidae) in Mississippi, USA

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Running Title: Leech Parasitism of Box Turtle *Terrapene carolina major*

Abstract

Ten leeches were collected from a Gulf Coast box turtle, *Terrapene carolina major*, found crossing a road in Gulfport, Harrison County, Mississippi, USA. Eight of the leeches were identified as *Placobdella multilineata* and 2 were identified as *Helobdella europaea*. This represents the second vouchered report of leeches from a box turtle. *Helobdella europaea* is reported for the first time associated with a turtle and for the second time from the New World.

Introduction

The first vouchered report of a leech parasitizing a box turtle was that of Richardson *et al*. (2016) who reported 14 individuals of *Placobdella multilineata* feeding on a Gulf Coast box turtle, *Terrapene carolina major*, collected in Gulfport, Harrison County, Mississippi in June 2015. Prior to the report of Richardson *et al*. (2016), the only previous report of leeches from a box turtle was that of Brown (1974), who reported that 7 of 169 (4%) Coahuilan box turtles, *Terrapene coahuila* collected in northern Mexico harbored 1 to 4 small unidentified leeches. *Terrapene coahuila* is the only truly aquatic box turtle (Brown 1974) although substantial aquatic behavior has been documented for *T. carolina* (Belusz and Reed 1969; Summers *et al*. 1998; McDowell *et al*. 2004; Donaldson and Echternacht 2005; Richardson *et al*. 2016). In two instances, Heaton (2017) observed Gulf Coast Box turtles swimming across a 100 m wide seaway in Gulfport, Mississippi. The purpose of this paper is to document the second vouchered report of leeches parasitizing a box turtle from Mississippi.

Materials and Methods

Leeches collected were prepared as described by Moser *et al*. (2006). Specimens were subjected to molecular analysis according to Richardson *et al*. (2010) as follows: Purified PCR products were sequenced using the HCO2198 primer and the LCO1490 primer (Light and Siddall 1999) for the Cytochrome c oxidase subunit I products by the W.M. Keck Foundation Biotechnology Resource Laboratory at Yale University. Aligned DNA sequences were compared to other leech DNA sequences contained within Genbank and in the authors' databases to confirm identifications and deposited in GenBank. Specimens of leeches were deposited in the Peabody Museum of Natural History at Yale University, New Haven, Connecticut (YPM IZ).

Figure 1. Gulf Coast box turtle, *Terrapene carolina major*.

Results

On 29 June 2016, 10 leeches were collected from a Gulf Coast box turtle, *T. carolina major*, found crossing East Taylor Road (30.413235°N, 89.024751°W), Gulfport, Harrison County, Mississippi, USA (Fig. 1). Eight of the leeches were heavily engorged with blood and were identified as *Placobdella multilineata* (YPM IZ 101900-101905) and 2 of the leeches were identified as *Helobdella europaea* (YPM IZ 101859) (Figs. 2,3). This constitutes the second report of *H. europaea* from the New World and the first report of *H. europaea* in association with a turtle.

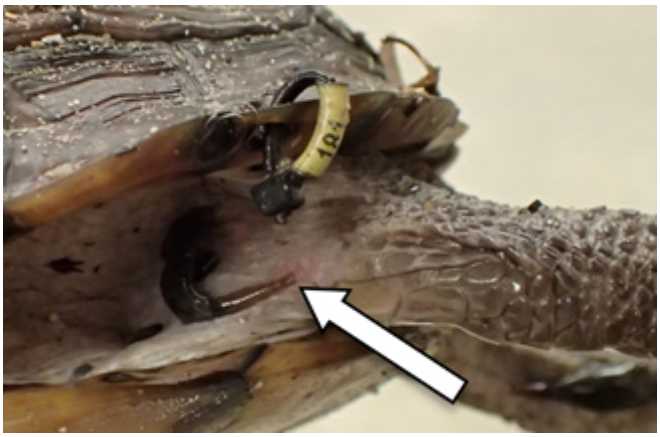

Figure 2. Individuals of *Placobdella multilineata* and *Helobdella europaea* (arrow) closely associated on the back left inquinal pouch of a Gulf Coast box turtle.

Figure 3. *Helobdella europaea* (YPM IZ 101859). Scale bar = 2 mm

Discussion

Placobdella multilineata is a generalist leech having been reported from 18 species and subspecies of alligators, amphiumas, crocodiles, snakes, and turtles from throughout the southeastern United States and northward through the Mississippi River valley to Illinois and Iowa (Moser *et al*. 2014; Richardson *et al*. 2016). This constitutes the second report of *P. multilineata* from a box turtle in Mississippi. Richardson *et al*. (2016) reported 14 individuals of *P. multilineata* from a Gulf Coast box turtle collected in Gulfport, Mississippi in June 2015. This second occurrence of these common parasitic leeches on a box turtle supports the assertion of Richardson *et al.* (2016) that *T. carolina* is not merely an incidental host, but rather a competent host for *P. multilineata*. Only 2 of 132 Gulf Coast box turtles examined from 2013 – 2016, including recaptures, were found to harbor leeches. In both the present report and that of Richardson *et al*. (2016) the leeches were collected from a turtle found crossing a road near water. Also, in both instances the turtles were collected during periods of hot weather in the summertime, a time during which these box turtles are occasionally observed inhabiting water (Fig. 4).

Figure 4. Gulf Coast box turtle, *Terrapene carolina major*, soaking in water.

Helobdella europaea was originally described by Kutschera (1985) as *Helobdella striata* from a fast-running stream in southern Germany. Upon discovery that the South American leech *Helobdella triserialis* var. *striata* preoccupied the name *striata*, Kutschera (1987) renamed the German species as *H. europaea*. Since its original description from Germany, *H. europaea* has been reported from Australia (under the junior synonym *Helobdella papillornata*), New Zealand, South Africa, Hawaii, Taiwan, Germany, the Netherlands, Spain, Hungary and Alameda and Sacramento Counties in California, USA (Govedich and Davies 1998; Kutschera 2004; Siddall and Budinoff 2005; Bely and Weisblat 2006; Lai *et al*. 2009; Reyes-Prieto *et al.* 2013; Pfeiffer *et al.* 2014; Málnás *et al*. 2016).

Robust morphological and molecular analysis of the genus *Helobdella* led Siddall and Budinoff (2005) to conclude that species of the genus *Helobdella* originated

in South America and that reports of *H. europaea* from around the globe may have been a result of accidental introductions with common aquatic invasive plant species. This view has been widely accepted (Bely and Weisblat 2006; Lai *et al*. 2009; Reyes-Prieto *et al*. 2013). Furthermore, Siddall and Budinoff (2005) found that *H. europaea* is a sister species to *Helobdella triserialis sensu stricto* from Bolivia and that the *europaea/triserialis* cluster is sister to *Helobdella cordobensis* from Chile.

As pointed out by Siddall and Borda (2003), *Helobdella* spp. descended from ancestors that appear to have switched from being sanguivores to being predators of aquatic invertebrates with aquatic mollusks and oligochaetes being popular prey items (Siddall and Budinoff 2005). Although virtually identical molecularly, based on the COI analysis conducted by Siddall and Budinoff (2005), there is some discrepancy in the literature concerning feeding habits of *H. europaea*. Govedich and Davies (1998) reported that specimens of *H. europaea* from Australia feed exclusively on gastropod snails whereas specimens of *H. europaea* from Germany demonstrated to be more catholic in feeding habits, rapidly capturing prey items such as oligochaetes and sucking their body fluids with the aid of their proboscides (Kutschera and Wirtz 2001; Pfeiffer *et al*. 2004). In addition to aquatic snails, German *H. europaea* were reported to readily feed on oligochaetes (*Tubifex* sp.), insect larvae (*Chironomus* sp.), and isopods *(Asellus aquaticus*) (Kutschera 2004).

The current report represents the first report of *H. europaea* associating with a turtle. As pointed out by Richardson *et al*. (2017), leeches of the genus *Helobdella* are often encountered in low numbers on turtles (Readel *et al.* 2008, Davy *et al*. 2009) and it is generally accepted that these associations do not represent parasitism (Sawyer 1986; Siddall and Borda 2003; Richardson *et al*. 2010, 2015, 2017). It has been pointed out that the association of *Helobdella* spp. with turtles may be a manifestation of an ancestral physical association that may have been retained, especially if selective advantages are conferred by the association (Davey *et al.* 2009; Richardson *et al*. 2015). Access to prey, including other leeches (Sawyer 1972; Davey *et al.* 2009) is one such possible advantage. Richardson *et al*. (2017) commonly found individuals of *Helobdella octatestisaca* within clusters of young *Placobdella parasitica*, often attached to *P. parasitica*, on turtles in a Texas pond. This lead Richardson *et al*. (2017) to the hypothesis that *H. octatestisaca* was utilizing *P. parasitica* as a source of food and that *Helobdella* spp. may preferentially associate with turtles, thus providing enhanced access to their prey. Richardson *et al*. (2015) reported individuals of *Helobdella modesta, Helobdella papillata*, and *Helobdella lineata* from snapping turtles, stinkpot turtles, and painted turtles from Massachusetts and Connecticut, all of which are common hosts of *Placobdella* spp. The present finding of *H. europaea* along with *P. multilineata* on a box turtle is consistent with the hypothesis that *Helobdella* spp. may associate with turtles as a mechanism to provide enhanced access to prey.

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Persistence of Urban Stream Syndrome Effects from Point Source and Non-Point Source Pollutants

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Running Title: Persistence of Urban Stream Syndrome

Abstract

In a previous study, Sager Creek, a small 1st-3rd order stream in northwest Arkansas was shown to be negatively impacted by urban land usage within the watershed, producing a stream that exhibited several indicators of urban stream syndrome. This included (1) physical disturbances: increases in impervious surfaces in the watershed, dams built across the stream, and alteration of the natural stream flow through the construction of retaining walls, (2) chemical disturbances: increases in electrical conductivity (EC) and total dissolved solids (TDS) as well as elevated PO_4 levels (3) and biological disturbances: low populations of pollution intolerant macroinvertebrate species and high populations of pollution tolerant species. It could be hypothesized that these negative impacts could be mitigated by both biological and physiochemical remediation processes downstream from the effluent of the Siloam Springs Wastewater Treatment Plant (SSWTP), the most heavily effected of the previous study sites. A three-year investigation to test this hypothesis was completed. Utilizing physiochemical properties and biological assessments, four stream reaches, two in the previous research site and two downstream, were assessed for negative urban impact. Some acquired data supported the hypothesis that negative effects are mitigated downstream, particularly a lowering of EC and TDS levels and an increase in macroinvertebrate diversity. However, a larger amount of data, including mean water temperature, total water flow, pH, dissolved O_2 and NO_3 levels and mean Family-level Biotic Indices supported the null hypothesis that reaches above, at and, below the SSWTP were all equivalent in investigated physiochemical parameters and biological indicators.

Key words: stream macroinvertebrates, waste water effluent, water pollution

Introduction

Urban Stream Syndrome (USS) (Meyer *et al*. 2005; Walsh *et al*. 2005) is a term used to describe steam ecosystems that have been negatively affected by urbanization. Elevated levels of stream nutrients and contaminants, altered channel morphology, increases in pollution tolerant species and a corresponding decrease in biotic richness are all indicators of USS (Paul and Meyer 2001; Meyer *et al*. 2005).

In previous publications (Wakefield 2013; Wakefield 2014) it was revealed that the upper reaches of Sager Creek demonstrate USS as a result of altered stream geomorphology and both point and non-point sources of stream pollution. The introduction of pollutants into a stream or river initiates a series of negative effects in the downstream water. The nature of these effects could be physical, biological and/or chemical in nature (Bartsch 1948). Although these previous studies confirmed these negative effects for the upper reaches of Sager Creek, what has not been assessed is how far downstream these negative effects persist.

In a lotic system, with a clear point source of organic pollution, such as untreated waste water, a series of zones are predicted to be found downstream from the pollution source: a septic zone, in which concentrations of dissolved oxygen are reduced to zero by the biological oxygen demand (BOD) of microbes breaking down organic pollutants; a recovery zone where re-aeration of the stream water causes increasing levels of dissolved oxygen; and finally a clean water zone where the effects of the point source pollution can no longer be detected (Bartsch 1948). Depending on the amount of untreated water, and the size of the stream, the septic and recover zones could persist for miles downstream from the point source.

However, modern wastewater treatment plants are meant to serve as both the septic and recovery zones, and treatment plant effluent is assumed to be most closely associated with water in the clean water zone (Bartsch

1948). But it has been shown that even for modern wastewater treatment plants, effluent often contains many anthropogenic chemicals including inorganic and organic micropollutants such as artificial sweeteners, caffeine, and pharmaceuticals such as Erythromycin, Tramadol, and Codeine (Daughton and Ternes 1999; Dyer and Wang 2002; Englert *et al*. 2013; Cardenas *et al*. 2016). Thus, the assigning of wastewater effluent as "clean water", is overstated.

The purpose of this study was to utilize stream macroinvertebrate populations and physiochemical testing to determine if the water downstream from the SSWTP is truly in a "clean water zone", or if the waste water effluent produced persistent negative effects on the downstream reaches of Sager Creek. The null hypothesis for this study was that all reaches would show the same level of negative effects as a result of USS (Meyer *et al*. 2005; Walsh *et al*. 2005). However, according to Bartsch (1948), we could predict that the water chemistry and biota of the reaches downstream from the SSWTP effluent would show evidence of a healthy lotic system.

Materials and Methods

Sager Creek is a 21.6 km, (USGS 2016) 1-3 order stream (Vannote *et al*. 1980) located in an Ozark Highlands Ecoregion of Northwest Arkansas (Omernick 1987). The forty km^2 Sager Creek watershed includes pastures for grazing or hay production (55%), the urban area around the city of Siloam Springs (30.5%), and small "islands" of forest (11%). The primary "urbanized" areas are concentrated around the head waters of the creek, while pasture and forested areas dominate in the downstream reaches (AWIS 2006) The main channel of Sager Creek flows through the city of Siloam Springs, receives the waste water treatment effluent downstream from the city and continues to flow into Oklahoma where it becomes a tributary of Flint Creek, which eventually flows into the Illinois River.

The methods used for sampling in Sager Creek were outlined in a previous publication (Wakefield 2014). In brief, Sager Creek was sampled from September of 2013 until June of 2015. Four riffle-dominated reaches were sampled in the stream (Fig. 1). The first reach is found on the campus of John Brown University (JBU) which is upstream from the Siloam Springs Wastewater Treatment Plant (SSWTP), but downstream from the Siloam Springs urban area. The second reach begins where the SSWTP effluent enters the creek (WW), and proceeds downstream. A small bridge that crosses the stream, approximately 2.5 kilometers downstream from

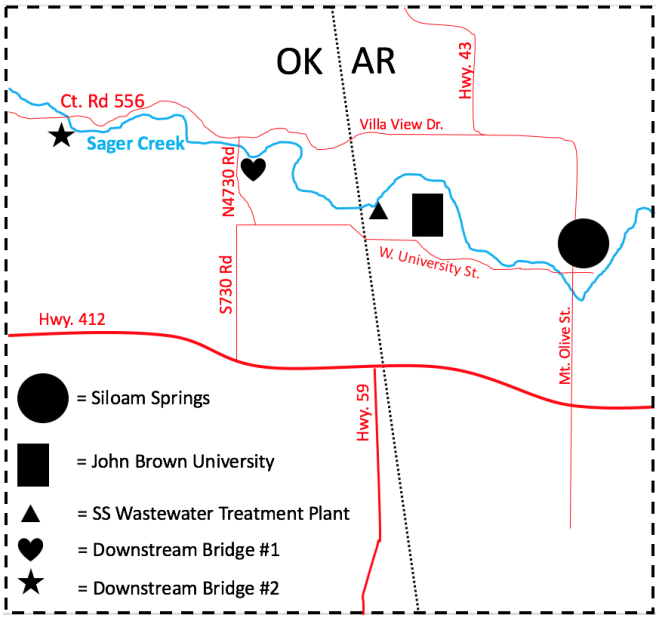

Fig 1. Map of Sager Creek indicating the location of the four sampled reaches.

the WW reach, was the location of the third reach, and was dubbed the downstream bridge #1 reach (DB1). Another small bridge crosses the stream, approximately 5 kilometers downstream from the WW reach, and was dubbed the downstream bridge #2 reach (DB2). Each sampling reach was divided into eight sampling sites, labeled A-H. During the three-year period, a total of 12 samples were collected from each reach, for a total of 48 separate samples (Table 1). Each sampling effort took approximately 3 hours to complete and one sample was collected per day. It should be noted that both the JBU and WW reaches are in Arkansas, while DB1 and DB2 are in the state of Oklahoma.

At each sampling site, organisms were captured in a 500-µm D-net. Net contents were poured through a 0.5 cm^2 mesh rock screen into a bucket. Both the D-net and the rock screen were inspected to remove all clinging organisms. The final sample was transferred into a collection container and preserved with 95% ethyl alcohol. All sampling sites were sampled in this same manner, with the exception of samples taken during May and June of 2015. Due to limited assistance and time, collections were made at only four of the eight sampling sites.

In the laboratory, each collected sample was poured into gridded counting tray and a subsample of 100 organisms was separated and identified to the family level (Needham and Needham 1962; Voshell 2002). A Hilsenhoff (1988) family-level biotic index (FBI) was generated from each subsample. This index utilizes 66

Table 1. Sampling dates for each reach sampled during 2013-2015.

JBU	WW	DB1	DB2
9/20/13	9/30/13	10/7/13	10/11/13
10/16/13	10/23/13	10/28/13	11/1/13
11/4/13	11/11/13	11/18/13	12/2/13
11/25/13	1/20/14	1/29/14	2/12/14
2/14/14	2/19/14	2/26/14	3/7/14
3/12/14	3/19/14	4/2/14	4/9/14
4/16/14	4/23/14	4/28/14	4/30/14
9/17/14	10/1/14	10/8/14	10/22/14
10/29/14	11/5/14	11/19/14	12/3/14
1/30/15	2/11/15	2/25/15	3/16/15
3/11/15	3/30/15	4/6/15	4/20/15
5/19/15	6/2/15	6/3/15	6/24/15

insect families, in 8 different orders, as well as 2 crustacean groups, (Isopoda and Amphipoda), to produce the FBI. In the FBI, streams with higher levels of organic pollution are designated with higher numeric values on a scale of 0 to 10. However, the Hilsenhoff's FBI was developed utilizing insects and crustaceans native to Wisconsin. Obviously, the arthropods in Sager Creek could have different tolerance levels. To better reflect these levels, organic pollution tolerance values, from 0-10, where 10 indicates the most tolerance, were assigned according to a database provided by the Missouri Department of Natural Resources (Sarver 2005).

The same subsamples from each site, were also used to develop a family-level Simpson's Index of Diversity (SID), (Simpson 1949). The SID is an indication of diversity within the stream. When stream diversity is high the probability increases that a second organism taken from the stream will be different from the first organism taken from the stream. The SID is calculated on a scale of 0-1 where 0 indicates that all organisms collected were in the same family, or there is no diversity, and 1 that indicates an infinite diversity of organisms.

A mean SID and mean FBI were calculated for each reach per sample day from the 8 individual site's SID and FBI. The 12 individual mean SID and FBI were recorded for each of the 4 reaches during the sampling period. To calculate a reach-specific mean SID (Reach Diversity) and reach-specific mean FBI (Reach Index), all twelve of the individual reach mean SID and FBI were utilized.

Additionally, all organisms from each of the 100 organism sub-samples, were used to produce a mean number of individuals from each arthropod family per reach (Family Mean). These values were useful to compare the overall diversity of pollution tolerant versus pollution intolerant species along the stream.

Sager Creek water flow was calculated utilizing Environmental Protection Agency (EPA) standard procedures (USEPA 2004). Stream temperature, pH, electrical conductivity (EC) and total dissolved solids (TDS) data were collected using a Hanna Instruments HI 991300 Multiparameter Water Quality Meter. Tests for concentrations of dissolved nitrate (NO_3), (cadmium reduction method 8039), phosphate (PO_4), (USEPA method 365.2), and dissolved oxygen (O_2), (HRDO method 8166), were performed on unfiltered water using a Hach™ colorimeter (model DR/850) according to EPA standard procedures (USEPA 2004). Each test was performed three times and a mean value for each parameter was calculated. Mean values for each parameter were then pooled in the same manner as Reach Diversity and Reach Index to produce a reach-specific mean (Reach Mean) for each parameter.

Student t-tests (α=0.05) were used to test for significant differences between Reach Diversity, Reach Index, Family Mean, and Reach Mean values between each Sager Creek reach.

Results

Physiochemical Parameters.--- Of the 8 physiochemical parameters tested, only levels of dissolved phosphate (PO_4), total dissolved solids (TDS) and electrical conductivity (EC) showed any significant differences. The student t-test analysis indicated that the JBU reach had lower levels of EC and TDS than all 3 downstream reaches. Student t-test analysis also indicated that the JBU reach had lower PO_4 levels than all three downstream reaches. However, the WW reach had a lower PO_4 level than the DB2 reach, and the DB1 reach had a significantly lower PO_4 level than the DB2 reach (Table 2).

Macroinvertebrate Diversity.--- The Reach Diversity of the JBU reach was statistically equivalent to both the DB1 and DB2 reaches. The diversity of macroinvertebrates in the WW reach, though, was statistically lower than all other reaches (Table 2).

As in the previous study (Wakefield 2014), all eight of the insect orders and the 2 crustacean groups were collected in this study. But only 31 of the potential 66 families were collected and used in creating both the

Table 2. Physiochemical & diversity parameters tested along Sager Creek. Student t-tests p-values are significant to the 95% confidence interval. Shaded boxes and bold text indicate significant results. ppm= parts per million; µS/cm= microsiemen per centimeter. n=12 per mean value.

Parameter	Reach Comparison X̄±SE		t-test
Reach Mean TDS (ppm)	JBU 152.25±6.14	WW 258.46±18.24	***p=3.15E-5***
		DB1 250.42±16.68	***p=2.67E-5***
		DB2 239.39±12.24	***p=8.19E-6***
	WW	DB1	nd
		DB2	nd
	DB1	DB2	nd

Parameter	Reach Comparison X̄±SE		t-test
Reach Mean PO_4 (ppm)	JBU 0.221±0.043	WW 0.405±0.093	***p=1.16E-2***
		DB1 0.457±0.048	***p=2.49E-4***
		DB2 0.532±0.062	***p=2.05E-5***
	WW	DB1	nd
		DB2	***p=3.68E-2***
	DB1	DB2	***p=2.68E-2***

Reach Mean EC (µS/cm)	JBU 304.61±12.33	WW 517.89±36.32	***p=3.00E-5***
		DB1 501.69±33.52	***p=2.84E-5***
		DB2 478.86±24.42	***p=8.00E-6***
	WW	DB1	nd
		DB2	nd
	DB1	DB2	nd

Reach Diversity	JBU 0.762±0.026	WW 0.574±0.052	***p=1.94E-4***
		DB1 0.724±0.041	nd
		DB2 0.711±0.043	nd
	WW	DB1	***p=2.66E-3***
		DB2	***p=2.58E-2***
	DB1	DB2	nd

Reach Mean Temp (°C)	nd
Reach Mean NO_3 (ppm)	nd
Reach Mean Water flow (m^3/s)	nd

Reach Index	nd
Reach Mean O_2 (ppm)	nd
Reach Mean pH	nd

Reach Diversity and Reach Index. Table 3 indicates that three families of Ephemeroptera, one family of Plecoptera, 3 families of Trichoptera, and one family each of Odonata, Diptera and Coleoptera showed significant results. All other insect families and crustacean orders showed no significant differences.

For the Ephemeropterans, all 3 families showed significant t-test differences. For the family Baetidae, statistical differences were noted between the JBU reach and the DB1 and DB2 reach. This family also showed a significant difference between the WW reach and DB1 and DB2 reach. The family Isonychiidae showed the same significant differences in reaches as was seen in the family Baetidae. For the family Leptophlebiidae, the only significant differences were seen between the JBU reach and the DB1 and DB2 reaches.

For the Trichopterans, all three families also showed significant t-test differences. Philopotamidae showed differences between all reach comparisons except for the comparison between DB1 versus DB2. Hydropsychidae also showed significant differences in every comparison except between JBU versus DB2. The Helicopsychidae were only found in small numbers at two of the reaches. This resulted in significant differences between only the JBU reach and both the WW and DB2 reach.

The Plecopteran family Perlidae, was also found in limited numbers and they were all at the downstream bridge reaches. This resulted in significant t-test differences in all comparisons except for the JBU versus

Table 3. List of orders and families of aquatic insects and crustacean taxa collected, identified, and counted in Sager creek. Numbers at the end of each taxon indicates the pollution-tolerance value according to Sarver (2005). Student t-tests p-values are significant to the 95% confidence interval. Shaded boxes and bold text indicate significant results. n=12 per mean value.

Macroinvertebrate	Family Mean $\bar{x}$±SE		t-test
Ephemeroptera	JBU 40.92±7.85	WW 38.33±14.95	nd
Baetidae(4)		DB1 133.41±28.03	***p=1.06E-3***
		DB2 122.25±23.90	***p=1.06E-4***
	WW	DB1	***p=1.06E-5***
		DB2	***p=1.06E-6***
	DB1	DB2	nd
Leptophlebiidae(2)	JBU 4.75±1.97	WW 1.08±0.69	nd
		DB1 0.25±0.18	***p=2.07E-2***
		DB2 1.58±1.02	***p=3.48E-2***
	WW	DB1	nd
		DB2	nd
	DB1	DB2	nd
Isonychiidae(2)	JBU 2.08±0.91	WW 1.25±0.79	nd
		DB1 10.67±4.41	***p=4.37E-2***
		DB2 13.25±5.36	***p=2.96E-2***
	WW	DB1	***p=3.32E-2***
		DB2	***p=2.57E-2***
	DB1	DB2	nd
Caenidae(7)	nd		
Heptageniidae(4)	nd		
Leptohyphidae(4)	nd		
Ephemerellidae(1)	nd		
Ephemeridae(4)	nd		
Odonata	JBU 13.08±2.37	WW 34.92±11.63	***p=2.88E-2***
Coenagrionidae(9)		DB1 16.00±5.21	nd
		DB2 7.25±1.90	***p=1.53E-2***
	WW	DB1	***p=1.12E-2***
		DB2	***p=1.69E-2***
	DB1	DB2	nd
Calopterygidae(5)	nd		
Gomphidae(7)	nd		
Libellulidae(9)	nd		
Diptera	JBU 0.25±0.13	WW 0.00	***p=4.09E-2***
Tabanidae(8.5)		DB1 0.00	***p=4.09E-2***
		DB2 0.00	***p=4.09E-2***
	WW	DB1	nd
		DB2	nd
	DB1	DB2	nd
Ceratopogonidae(6)	nd		
Chironomidae(6)	nd		
Empididae(6)	nd		
Simuliidae(6)	nd		
Tipulidae(3)	nd		

Macroinvertebrate	Family Mean $\bar{x}$±SE		t-test
Trichoptera	JBU 151.75±19.8	WW 9.75±2.00	***p=1.18E-5***
Philopotamidae(3)		DB1 60.33±20.61	***p=3.78E-3***
		DB2 40.5±8.28	***p=4.10E-5***
	WW	DB1	***p=1.31E-2***
		DB2	***p=9.95E-4***
	DB1	DB2	nd
Hydropsychidae(4)	JBU 89.83±12.43	WW 183.41±57.64	***p=3.72E-2***
		DB1 48.66±13.37	***p=6.62E-4***
		DB2 72.42±13.52	nd
	WW	DB1	***p=7.37E-3***
		DB2	***p=2.86E-2***
	DB1	DB2	***p=1.45E-2***
Helicopsychida(3)	JBU 0.66±0.31	WW 0.00	***p=2.72E-2***
		DB1 0.16±0.11	nd
		DB2 0.00	***p=2.72E-2***
	WW	DB1	nd
		DB2	nd
	DB1	DB2	nd
Hydroptilidae(4)	nd		
Limnephilidae(3)	nd		
Polycentropidae(6)	nd		
Plecoptera	JBU 0.00	WW 0.00	nd
Perlidae(3)		DB1 1.83±0.44	***p=7.98E-4***
		DB2 4.00±1.20	***p=3.44E-3***
	WW	DB1	***p=7.98E-4***
		DB2	***p=3.44E-3***
	DB1	DB2	***p=4.28E-2***
Capniidae(1)	nd		
Coleoptera	JBU 38.75±7.74	WW 24.92±6.02	***p=2.72E-2***
Elmidae(4)		DB1 76.42±14.38	***p=5.62E-3***
		DB2 111.33±36.49	***p=3.17E-2***
	WW	DB1	***p=5.27E-4***
		DB2	***p=1.11E-2***
	DB1	DB2	nd
Psephenidae(4)	nd		
Lepidoptera			
Pyralidae(5)	nd		
Amphipoda(6.9)	nd		
Isopoda(8)	nd		

WW reach.

For the Coleopterans, the family Elmidae were found in all of the reaches sampled and significant t-test differences were noted for all comparisons except for the DB1 versus DB2 comparison.

The one family of Odonata, Coenagrionidae, was also collected at all the reaches, but the JBU reach showed significant t-test differences between both the WW and DB2 reaches. The WW reach also showed significant differences between both DB1 and DB2 reaches.

Although many different families of Dipterans were collected, the only family that showed any significant differences were the Tabanidae. This family was only collected at the JBU reach and was thus significantly different from all other compared reaches.

Discussion

According to Wakefield (2014), the upper reaches of Sager Creek show a significant amount of USS from the urban setting surrounding the stream, including altered geomorphology, altered water chemistry and altered biota. One of the most significantly affected reaches is the WW reach presumably from the negative impact of the SSWTP effluent. But, according to Bartsch (1948), this effluent should represent water that has already been through the septic zone and the recovery zone while in the treatment plant. Therefore, although the effluent may show a significant impact on overall stream health, the persistence of the impact should be relatively short-lived in the downstream reaches of the stream and the overall stream health should recover to the pre-effluent level (as represented by the JBU reach) or could even fully recover to a "clean water" level as it progresses downstream.

***Physiochemical Parameters*---**The physiochemical symptoms of USS were inconsistent among the four Sager Creek reaches. Five of the eight parameters tested confirmed the null hypothesis, as there were no significant differences found between any of the reaches (Table 2). However, both TDS and EC showed significant statistical differences. This is not surprising considering that a previous study had already identified the WW reach as a point source for elevated TDS (Wakefield 2014) and that elevated EC is directly correlated with elevated TDS, (MacPherson 1995). Table 2 indicates that there is a rapid increase in concentration of TDS and EC at the WW reach and that both slowly decline the farther downstream the water progresses. This pattern is predictable and conforms to expectations of effluent released pollutants (Fono *et al*. 2006; Paul and Meyer 2001).

The effluent from a wastewater treatment plant can also be a significant source of dissolved PO_4 (LaValle 1975). Significant levels of dissolved PO_4 have already been demonstrated to be a major component of the SSWTP effluent (Haggard *et al*. 2004; Wakefield 2014). What is curious is that the level of dissolved PO_4 continues to increase as the water moves downstream (Table 2). This could be an indication that additional non-point sources of PO_4 are being added to the stream. This is a strong possibility as the downstream watershed is dominated by agricultural pasture and grazing land that could be leaching dissolved PO_4 into the stream (Sharpley and Sharpley 1994).

Biological.--- Additional "mixed" results are seen in the biological studies performed. Although the Reach Index showed no significant differences, Reach Diversity showed significant statistical difference (Table 2). In general, macroinvertebrate diversity is negatively correlated with stream pollution levels (Pratt *et al.* 1981; Hachmoller *et al.* 1991; Thorne *et al*. 2000). The JBU Reach Diversity was significantly higher than the WW reach but not the DB1 or DB2 reaches. The WW reach was significantly lower than both the DB1 and DB2 reaches. However, the DB1 and DB2 were not significantly different from each other. This pattern is predictable, if it is assumed that the downstream reaches are approaching pollution levels on par with the pre-effluent effected stream water.

Of the thirty-one insect families and Crustacean Orders collected, twenty-one showed no statistical difference (Table 3). For those families that did show significant differences, the t-test results of compared reaches are still problematic. For example, the Ephemeroptera, Plecoptera and Trichoptera (EPT) orders are typically thought of as being the most pollution sensitive. Thus, based on the Reach Diversity results, it could be predicted that the families of these three orders would show similar population levels in the JBU reach, DB1 reach and DB2 reach if the water quality is approaching the pre-effluent effected level. Alternatively, if the water quality is approaching a higher "clean water" stage then the DB1 and DB2 reaches might have even greater population levels than either the JBU or WW reach. For some of the EPT families these "expected" results are seen. This was true for the families Baetidae, Isonychiidae and Perlidae. The Coleopteran family Elmidae also reflects these expected results. However, for the families Leptophlebiidae, Philopotamidae and Helicopsychidae the JBU reach showed the highest population levels. This was also true for the Dipteran family Tabanidae.

What is not surprising is that the Odonate family Coenagrionidae shows a significantly higher population

in the WW reach. This is predictable considering that the Coenagrionidae have one of the highest pollution tolerance levels. What is surprising, though, is that the Trichopteran family Hydropsychidae, with a low to mid-range tolerance level, also reaches its significantly highest population level in the WW reach.

These mixed results amongst the macroinvertebrate families could be reflective of the mixed results seen in the physiochemical characters. For example, the WW, DB1 and DB2 reaches were demonstrated to have significantly higher levels of TDS, EC and PO_4. It is possible that the families Leptophlebiidae, Philopotamidae and Helicopsychidae are particularly sensitive to one or more of these parameters, thus reducing their numbers downstream from the JBU reach. Whereas the families Baetidae, Isonychiidae and Perlidae may not be particularly sensitive to any of these parameters, and the pollutant that prevents them from flourishing in either the JBU or WW reaches is finally diluted away to a suitable level in the downstream reaches. If this were true, the identity of that pollutant has not been elucidated in this or any other previous studies.

Conclusion

Although particular findings in the physiochemical parameters and biological assessments indicate that the four reaches studied along Sager Creek are significantly different, the large number of non-significant differences in biological and physiochemical parameters would make it imprudent to completely reject the null hypothesis that all Sager Creek reaches would show the same level of negative effects as a result of USS.

As a final note, the significant effect of the SSWTP effluent on the downstream reaches of Sager Creek cannot be overemphasized. As Bartsch (1948) stated, the plant should serve as both the septic and recovery zones before the release of effluent. During normal operating procedures the plant seems to fulfill this role well enough that some stream recovery is evident in the downstream reaches as is seen in some of the macroinvertebrate families studied.

However, shortly after data collection for this study concluded, a major biological "upset" occurred at the SSWTP. In late September of 2015, the Sager Creek Foods cannery, located in the downtown area of Siloam Springs, AR, had a power failure that resulted in a significant release of untreated wastewater into the SSWTP. Unprepared for this influx, the treatment plants effluent became septic. Dissolved oxygen levels observed downstream from the plant fell below 1 mg/L (Smoot 2015). Warm water fish, such as *Lepomis cyanellus* (green sunfish), *L. macrochirus* (bluegill), and *Micropterus salmoides* and *M. dolomieu* (largemouth and smallmouth bass), require a dissolved oxygen level of approximately 5.5 mg/L (USEPA 1986), thus the resulting death of over 30,000 fish downstream of the plant. Although, the SSWTP is back to normal operating procedures (Myers 2016) the effluent from the plant will continue to pose a potential pollution risk for all the downstream reaches of Sager Creek.

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Ecology of Blanchard Springs Caverns, Ozark National Forest, Arkansas: 42 Years Later

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Running Title: Ecology of Blanchard Springs Caverns

Abstract

Interrelationships between subterranean and epigean environments affect dispersion and distribution of cave organisms among the macro and microhabitats. This study examined the environmental impact of 42 years of tourism and development in the two lower sections of Blanchard Springs Caverns found in Stone County, Arkansas; and contributes to a better understanding of the seasonal fluctuations of the abiotic and biotic parameters.

Temperature, water quality, and fauna data were collected. A new entrance, lighting, and approximately 12,500 visitors during the 12-month study had no observable effect on cavern temperatures. Stream water quality measurements were comparable to Grove's 1974 study. Gray bat, *Myotis grisescens*, populations and distributions increased from an estimated maximum of 5000 (Grove 1974; Grove and Harvey 1974) to 372,726 reported by U.S. Forest Service (personal communication, Jessica Hawkins, Sylamore District of the Ozark National Forest, Mountain View (AR), 2016). This study reported 5 obligate cave species all recorded in previous studies.

Introduction

Blanchard Springs Caverns is a limestone cave system located in the Sylamore Ranger District of the Ozark-St. Francis National Forest, which is 25 km northwest of Mountain View, in Stone County, Arkansas. It is the second largest cave in Arkansas, with 13.7 km in mapped length (Graening *et al.* 2011) and a delineated recharge area of 39.6 sq. km [15.3 sq. miles] (Aley 1980).

The U.S. Forest Service administers 3 guided tours for the public on the Dripstone Trail, the Discovery Trail and the Wild Cave Trail. The Dripstone Trail opened to the public in 1973 and is open all year round. It is approximately 1.6 km long and its largest room is 55 m wide and 366 m long. The Discovery Trail lies below the Dripstone Trail and is 1.9 km long and averages approximately 100 m underground. The Discovery Trail opened to tourist in 1977 and is open June through August. It includes a natural pit-entrance, an underground stream, which exits lower in the valley as Blanchard Springs, and exits from the Ghost Room. The Wild Cave Trail extends beyond the Discovery Trail and continues into undeveloped portions of the cave to the farthest point easily accessed by visitors. Access to the Wild Cave Trail is through the Ghost Room door. It is 2 km long, opened to tourist in 2000, and is open from April to October. The seasonal schedules of the Discovery Trail provide protection to bats hibernating in the lower sections of the cave during the winter months.

Blanchard Spring Caverns is a living cave, meaning speleothems are actively forming and undergoing change due to calcite deposition and dissolution. Monitoring abiotic and biotic factors of such a dynamic environment is essential for successful cave management. It is generally assumed that caves are characterized by relatively stable internal microclimates (Mohr and Poulson 1966); however, such cave ecosystems are not typically subjected to tourism and development, which have the potential to alter temperature, relative humidity and cave airflow. Altered airflow may have a greater impact on a cave environment than cave visitors. Hypothetically, during the warmer months, surface temperatures and relative humidity are generally high. This warm moist air may be drawn into the cave driven by convective airflow and differences in elevations of entrances. When this warm, moist, surface air is cooled by cooler cave temperatures, condensation occurs. In the cooler months, when surface temperatures and relative humidity are generally lower than cave temperatures, no condensation occurs and drying may occur. Aley

and Aley (1978) measured air flow exchanges in Blanchard Springs Caverns between the surface and cave as high as 1415.8 cubic meters [50,000 cubic feet] per minute when all cave doors were opened. They further reported that this air was capable of drying the cave 80% of the time. The reasons attributed to such high air exchanges were the larger size of the caverns, the 120 meters [240 feet] vertical extent of the cave between the elevator shaft and natural entrance, and the numerous entrances for air to pass.

In the winter of 1972, 6 months prior to the Dripstone Trail opening to the public, and prior to the development of the Discovery Trail, Grove (1974) conducted a baseline ecological study of Blanchard Springs Caverns. Data for temperature, humidity, water hardness, alkalinity, and cave fauna were recorded. At that time, temperatures near the entrance fluctuated between -7°C and 15°C, but generally remained a constant 14.5°C deeper in the cave. Relative humidity was generally 100%. The total alkalinity and total hardness of the cave stream fluctuated from 68-137 ppm to 111–205 ppm, respectively (Grove 1974). Aley and Aley (1978) analyzed data between June 1972 and January 1977 during the period of new construction of the lower Discovery Trail. They found that, between the natural entrance and the new tunnel into the Ghost Room, average temperatures fell 0.78 degrees C [1.4 degrees F] and mean relative humidity fell 2.7%.

The current study duplicates Grove's original 1974 study of the lower sections of Blanchard Springs Caverns and provides additional temperature and relative humidity data to the Aley and Aley (1978) meta-analysis. The current study determines the impact of 42 years of tourism and whether a more stable environment has been reestablished following the development of the Discovery Trail and Wild Cave Trail sections of the cavern. This research is of special importance because Blanchard Springs Caverns appears to be a major winter hibernaculum for endangered gray bats, *Myotis grisescens*, as well as other rare cave organisms. Graening *et al*. (2011) ranked [Blanchard Springs Caverns] as the second highest cave in Arkansas for biodiversity and as the most biologically important cave in Arkansas by number of rare species.

Materials and Methods

This study took place from June 2015 to May 2016. Humidity, water hardness, alkalinity, and cave fauna observations were obtained quarterly. Temperature sensors were placed (Smart Button Temperature Loggers, ACR Systems, Inc., Surrey, British Columbia, Canada, model ACRSB) in similar locations to the Taylor maximum/minimum thermometers from Grove's original study. The constant temperature zones, bat hibernacula areas, small rooms frequented by cave visitors, and the bottom of the natural pit entrance were of special interest. Additional sensors were placed in the constant temperature zone of the Wild Cave Trail and near the new artificial entrance and passages in the Ghost Room. Additionally, sensors were placed outside the artificial entrance of the Ghost Room to record epigean temperatures. The sensors were programmed to log temperatures every 90 minutes, for 90 days, at which time they were exchanged. Relative humidity was obtained using a sling psychrometer during the quarterly visits. Water quality was measured quarterly using titration field test kits (Hach Company, Loveland, Colorado, models OX-2P; 5-EP; and AL-AP-MG-L) for dissolved oxygen, total hardness, and alkalinity. Graening *et al*. (2003) completed extensive faunal surveys; therefore, no organisms were removed from the cave for the current study. Cave faunal observations were conducted during visits to retrieve temperature sensors and by cavern tour guides between research visits. Sterilized horse manure was used as bait in Petri dish traps. Any organisms observed were identified, photographed, and immediately released. Daily tourist visitation numbers, dates, and yearly bat population numbers were obtained from the U.S. Forest Service.

Results

During this study, 12,493 visitors toured the Discovery Trail and the Wild Cave Tour (personal communication, William Avey, U.S. Forest Service, 2017). More specifically, 11,990 visitors toured the Discovery Trail over 92 days on 431 tours, which were at 73.8% capacity. The average number of visitors per tour was determined to be 28 and each of these tours lasted approximately 1.5 hours. This calculates to 17,985 visitor-hours in the cave, with an average visitor-hour per tour of 41.73 (Sasser 2016).

During the study, 503 visitors toured the Wild Cave Trail over 82 days on 83 tours, which were at 74.4% capacity (Avey 2017). The average number of visitors per tour was found to be 6 and lasted approximately 5 hours. This calculates to 2515 visitor-hours with an average visitor-hour per tour of 30.3 (Sasser 2016).

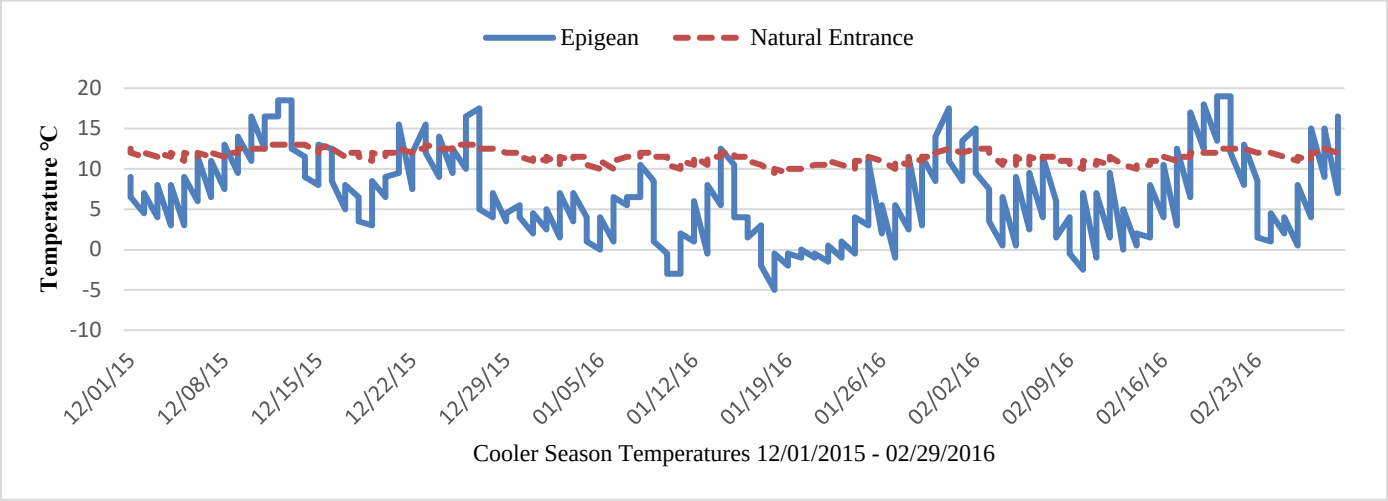

Figure 1. Comparison of epigean and natural entrance cooler season temperatures.

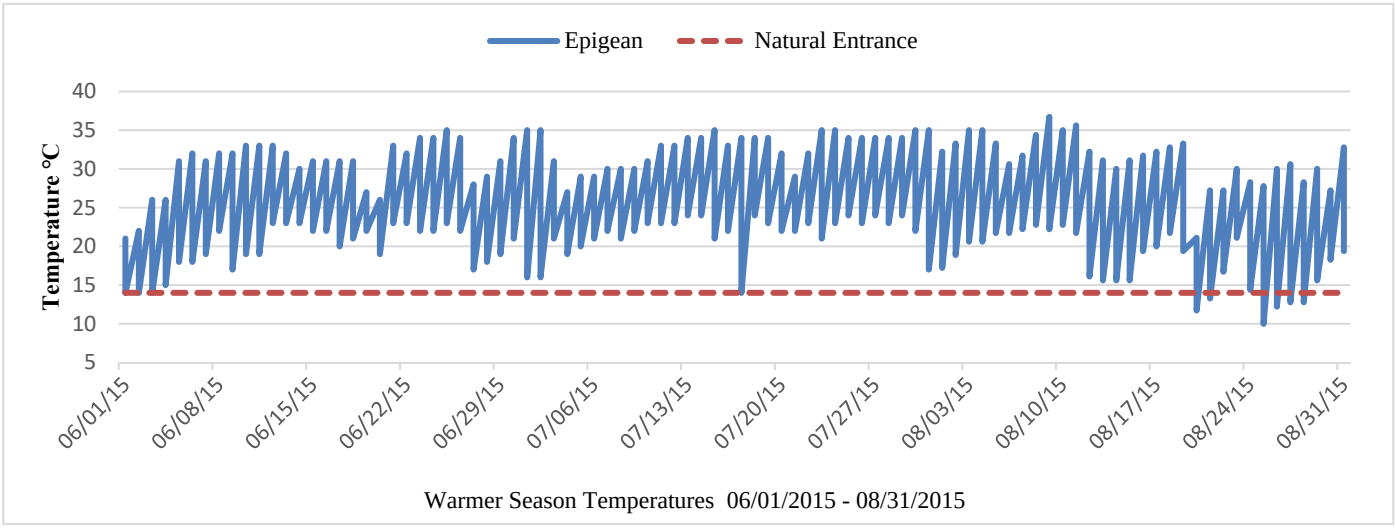

Figure 2. Comparison of epigean and natural entrance warmer season temperatures.

The researchers observed no temperature changes that could be attributed to visitor presence. The physical changes to accommodate tourism appear to have had no negative impact on temperature in the cave. The current study's cavern temperatures varied between 9.5°C to 14°C. The area near the natural entrance had the greatest variation in temperature during the cooler months, 9.5°C to 12.5°C (See Figure 1). During the warmer months, the temperature remained 14°C (Figure 2).

In the deepest cave zones, temperatures remained 14°C. Temperature sensors can only resolve 0.5°C; therefore, temperature fluctuations of 0.5°C were considered acceptable (*personal communication*, Eric Durand, eric@acrsystems.com, ACR, 2017).

A passage room, called the Reed Rock Room, was of special interest because it was a small area approximately 150 m^3 where visitors would congregate to listen to tour guides. If the 17,985 cavern visitor-hours (Sasser 2016) affected cave temperatures, it would be expected to be recorded in this room; however, temperatures did not vary from 14°C throughout the collection period. Additionally, temperatures in the Ghost Room, adjacent to the artificial entrance, remained 14°C. Stream quality measurements of dissolved oxygen ranged from 8-10 ppm. Alkalinity ranged from 95-135 ppm. Total Hardness ranged from 153-171 ppm. Stream quality measures fell within similar ranges as reported by Grove (1974) (Table 1).

Table 1. Stream Quality Comparison 1974-2016

	1974	2016
Dissolved Oxygen	9.5-10.5 ppm	8-10 ppm
Alkalinity	68-137 ppm	95-135 ppm
Total Hardness	111-205 ppm	153-171 ppm

Cave fauna observed were 5 obligate, troglobitic species including cave millipedes, cave spiders, pseudoscorpions, diplurans, and grotto salamanders, *Eurycea spelaea*. Other species observed included troglophiles, cave salamanders, cave crickets, gray bats, *Myotis grisescens*, northern long-eared bats, *Myotis septentrionalis*, and Indiana bats, *Myotis sodalis*. The *Myotis grisescens* winter population during the study was estimated to be 372,726 (U.S. Forest Service 2016).

Discussion

Tourist visitation did not have a measurable effect on temperatures in the sampled areas. The artificial entrance did not affect temperatures in the adjacent Ghost Room. This is most likely due to the presence of airlock doors and limited visitors relative to the volume of the cave passages.

Blanchard Springs Caverns made significant changes through the years to accommodate tourism in the lower sections of the cave, including a new tourist entrance, numerous concrete walkways, and incandescent lighting. The lack of any measurable detriment to the caverns resulting from such modifications is most likely attributed to the conservation efforts on the part of the U.S. Forest Service.

The U.S. Forest Service has many procedures in place that contribute to the preservation of the caverns. For example, airlock doors at entrances control changes in airflow; daily tour numbers and tour size are limited; lights are turned off, after tourists have left the area, to control heat and algal growth; and visitors on the Wild Cave Tours are required to wear clean cave clothing provided by the U.S. Forest Service. In addition, they must thoroughly wash cave boots to prevent the spread of white-nose syndrome, *Pseudogymnoascus destructans*, a fungus that infects hibernating bats. Prior to embarking, and after returning from the Wild Cave Tour, visitors are required to change shoes or put on shoe coverings to minimize the spread of organics to new areas of the cave and other caves.

In the initial base line study, Grove (1974) reported 36 taxa composed of 7 troglobites, 3 troglophiles, 12 trogloxenes, and 14 accidentals. Animal groups represented included rotifers, gastropods, isopods, amphipods, pseudoscorpions, spiders, millipedes, centipedes, collembolans, cave crickets, flies, diplurans, amphibians, birds, and mammals. Graening *et al*. (2004) reported Blanchard Springs Caverns complex as the "most species rich cave in Arkansas" with 96 taxa. Graening *et al*. (2011) later reported 126 taxa including 11 species of bats. The current study reported five obligate species, all previously reported. An increase in the number of fauna is most likely due to the time and effort of many cave scientists in the last 42 years and the decision of managers to allow accessibility for scientific research.

Populations and distributions of gray bats, *Myotis grisescens*, were found to have steadily increased from an estimated maximum of 5000 (Grove 1974; Grove and Harvey 1974) to 372,726 reported by U.S. Forest Service (2016). The increase in populations is most likely due to the favorable conditions Blanchard Springs Caverns provides during winter hibernation.

Conclusions and Recommendations

Tourism and development does not appear to have affected Blanchard Springs Caverns adversely as evidenced by the limited abiotic changes recorded and the growing number of fauna reported in the last 42 years. The temperature and relative humidity increases reported by Aley and Aley (1978) during the construction in the lower passages between the natural entrance and Ghost Room appear to have reestablished to pre-development levels reported by Grove (1974).

The dramatic increase in the gray bat population is especially significant. Blanchard Springs Caverns may have displaced significant caves on U.S. Forest Service land as the major winter hibernaculum. The authors recommend that research be undertaken to establish if this has occurred.

The U.S. Forest Service tour guide personnel are an integral part of the conservation and quality of tourism experienced while caving in Blanchard Springs Caverns. The authors recommend that an identification of cave fauna pamphlet and fauna inventory card be developed that could be used to assist tourists in becoming "citizen scientists" while in the cavern on the Wild Cave Trail. This would aid in recording fauna and educating tourist of the importance of fragile cave ecosystems. The authors also believe it would promote the beauty and educational benefit of Blanchard

Springs Caverns to the public.

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This paper is dedicated to the memory of Dr. Michael (Mick) J. Harvey.

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Local Scale Comparisons of Avian and Woody Vegetation Communities within Four Arkansas State Parks

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Running Title: Comparison of Biotic Communities in 4 Arkansas State Parks

Abstract

Measuring the spatial distribution of biotic communities can provide useful data to wildlife managers on how and why species assemblages differ across a landscape. During 18 May – 7 August 2015, we conducted avian point counts and collected vegetation data in nested subplots at 4 Arkansas state parks. We then used a series of one-way ANOVAs and Kruskal-Wallis tests to examine differences in species richness, Simpson's evenness, Simpson's diversity, and Bray-Curtis similarity across the 4 parks. Mount Magazine State Park had the lowest avian evenness ($F_{3,22} = 9.57$ $P = 0.003$) and diversity ($F_{3,22} = 17.8$ $P \leq 0.001$). Mount Magazine also had the lowest understory vegetation evenness ($F_{3,22} = 9.41$ $P \leq 0.001$) and diversity ($F_{3,22} = 17.8$ $P \leq 0.001$). Our analyses provided weak evidence supporting a possible relationship between avian and understory woody vegetation communities at Mount Magazine; however, this relationship was not observed in the remaining parks. Comparing biotic communities across 4 local state parks may aid park managers by providing a baseline of biotic data that can be used to better understand the collective effects acting on a specific park's flora and fauna.

Introduction

Biodiversity can be measured within a mosaic of spatial scales, with biotic communities often governed by a mix of both local and regional processes (Turner *et al*. 1989; Noss 1990; Huston 1999; Atauri and de Lucio 2001; Agrawal *et al*. 2007; Harrison and Cornell 2008). Patterns of biodiversity may also differ depending on the spatial scale of observation (Scrosati and Heaven 2007; Marsh and Trenham 2008). Understanding the influences acting on biotic community structure and how those communities and influences change across spatial scales is imperative for the management of flora and fauna in protected areas.

Research on the influence of external factors on biotic communities has been conducted primarily at 2 spatial perspectives: the regional scale and the local scale (Caley and Schluter 1997; Hillebrand and Bleckner 2002; Harrison and Cornell 2008; Hillebrand *et al*. 2008). Studies at the regional scale typically research species populations across states, biogeographic regions, or continents (Ricklefs 2004; Harrison and Cornwell 2008). Studies at the local scale focus on community influences to the extent of an individual site or cluster of sites (Huston 1999; Harrison and Cornell 2008). Biotic community structure at the regional scale is shaped by long-term, historic changes in habitat (i.e., geology, climate, historic land use), while local scale structure can be attributed to daily changes in weather, availability of resources, and alterations to habitat structure and use by protected area managers (Böhning-Gaese 1997; Ricklefs 2004; Harrison and Cornwell 2008).

State parks serve as a primary setting for local scale studies, in that biotic communities within state parks may differ from neighboring parks due to local differences in habitat structure and resource availability due to differing park management strategies. The likelihood of human-wildlife interaction changes throughout state parks, depending on the location and frequency of human activities and the distribution of wildlife (Cole 1993; Leung and Marion 2000). For example, parks that offer longer hiking trails that bisect a greater variety of natural habitats may have increased human-wildlife interactions compared to parks that have shorter trails or that have stronger restrictions on park use (Torn *et al*. 2009). Differences in vegetation structure and resource availability may further change depending on the habitat structure within the park as well as what the conservation objectives are for each park (Cueto and Casenave 1999). By focusing research among clusters of neighboring state parks, there is a potential to examine the influences shaping local community biodiversity within those state parks.

Our goal was to quantify and compare local avian and woody vegetation communities across 4 state parks in central Arkansas. Providing baseline community metrics for state park flora and fauna while simultaneously observing how these communities differ across neighboring parks may aid managers in mitigating the effects of human recreation and park management that have shaped the species composition and communities within those parks.

Methods and Materials

Four state parks located in close proximity to the Arkansas River in central and west-central Arkansas served as the focus for our study: Mount Magazine State Park, Petit Jean State Park, Mount Nebo State Park, and Pinnacle Mountain State Park. Mount Magazine, Mount Nebo, and Petit Jean State Parks are located in the Arkansas River Valley ecoregion and Pinnacle Mountain State Park is located in the Ouachita Mountain ecoregion (USEPA 2016).

Mount Magazine State Park is located in Logan County, south of Paris, AR (15 S 442199, 38952229) and encompasses 904ha surrounded by the Ozark National Forest. The park is positioned on top of Mount Magazine (839m), a flat-topped plateau rimmed by sandstone bluffs. Compared to the other parks in this study with smaller elevations, Mount Magazine is locally considered "montane" and the diverse collection of wildlife and vegetation species reflects this habitat description. Average temperature for Mount Magazine during the study was 23.0°C with a mean precipitation of 7.26mm.

Mount Nebo State Park is located in Yell County, west of Dardanelle, Arkansas (15 S 476945, 3897552) and encompasses 1,246ha of habitat. The park is centered on top of Mount Nebo, which measures 411m in elevation. The habitat is mostly comprised of thick oak (*Quercus* spp.) and hickory (*Carya* spp.) dominated forests, characteristic of the Ozark Plateau region, with mixes of sweetgum (*Liquidambar styraciflua*) and red maple (*Acer rubra*) stands throughout the park. Average temperature for Mount Nebo during the study was 26.7°C with a mean precipitation of 8.33mm.

Petit Jean State Park is located in Conway County, west of Oppelo, Arkansas (15 S 505957, 3886563). Petit Jean mountain (368m) lies between the Ozark and Ouachita mountain ranges in the Arkansas River Valley and serves as the midpoint for the 1,416ha park. The habitat is comprised mostly of forests dominated by a mix of oak, hickory, and pine (*Pinus* spp.) stands within a series of ponds, streams, and glades, also characteristic of the Ozark mountain ecoregion (USEPA 2016). Average temperature for Petit Jean during the study was 26.4°C with a mean precipitation of 1.87mm.

Pinnacle Mountain State Park is located in Pulaski County, Northwest of Little Rock, Arkansas (15 S 547062, 3855665) and encompasses 809ha surrounding Pinnacle Mountain (308m). The park is composed of a mosaic of habitats including boulder fields, bald cypress (*Taxodium distichum*) swamps, bottomland hardwood forests, and upland forests composed of mixes of oak, hickory, and pine stands. The park's habitat includes an Arboretum that contains woody vegetation from across the state and the Big and Little Maumelle rivers that transect the park. Average temperature for Pinnacle Mountain during the study was 28.9°C with a mean precipitation of 0.49mm.

During 18 May – 7 August 2015, we sampled avifaunal and woody vegetation communities in cyclic 1-week increments. We rotated among the 4 parks so that each park was sampled 3 times during the study. Sampling took place on trails chosen within each park based on total trail length, diversity of habitat types that a trail traversed, and the total area each trail encompassed within the park. We included all trails measuring ≤ 16km in length and split trails measuring 8 – 16km into 2 equal portions to accommodate temporal limitations. We used ArcGIS (Environmental Systems Research Institute, Inc., Redlands, CA) to assess the diversity of habitat types represented along each trail (USEPA 2016) and the total area of the trails within each park. Applying these criteria resulted in 26 trails included in the study, with 6 trails each at Mount Magazine State Park, Mount Nebo State Park, and Petit Jean State Park and 8 trails at Pinnacle Mountain State Park. Initial sampling locations for avian point counts and vegetation subplots along trails were located randomly within the first 250m of each trail's trailhead. Subsequent sampling locations were then systematically located every 250m to ensure independence of bird count data (Ralph *et al*. 1995; Torn *et al*. 2009).

Avian point counts began ≤15min of sunrise each weekday and lasted approximately until 5 hours after sunrise. Point counts lasted 5-min each with birds sighted/heard at each 50m-radius point identified to species level and specified in their location to the study point, their distance from the study point, and whether the record was visual or auditory via symbols established by Ralph *et al*. (1993). We conducted point counts only during suitable weather conditions for avian activity defined as mornings with no rain or fog (Cyr *et al*. 1995; Martin *et al*. 1997); wind speeds <13km/hr (Freedmark and Rogers 1995; Petit *et al*. 1995); and

temperatures ranging 18 – 23°C (Buskirk and McDonald 1995; Martin *et al*. 1997).

Each avian point was sampled independently 3 times per week, once each by 3 observers (Petit *et al.* 1995). This methodology resulted in 9 visits for each of the 227 points (i.e., 3 times/week at each point during 3 independent weeks), with 45 minutes of total observation time collected per point. By utilizing 3 observers throughout the week rather than 1, as is common in many avian surveys, we were able to diminish repeated observer bias and increase the detection probability at each point (Ralph *et al*. 1995, MacKenzie and Royle 2005). Point counts along each trail were scheduled to prevent any point being visited at the same time throughout the week by any of the 3 observers.

We sampled woody vegetation subplots once at each sampling location during the study using a nested subplot method similar to James and Shugart (1970). Sampling occurred on adjusted points 16.3m off trail to establish a 5-m buffer between each trail edge and vegetation plot to avoid immediate edge effects (Brown *et al*. 2009). Subplots consisted of a 5-m radius plot, where we identified and counted all understory vegetation (saplings measuring ≤1.4m tall), nested in an 11.3-m radius plot, where we identified and counted all overstory vegetation (trees measuring >1.4m tall; Geldenhuys 1997, Rodewald and Brittingham 2004, Brown *et al*. 2009).

We calculated species richness (recorded as S), Simpson's Evenness Index, $E_{1/D} = \frac{(1/D)}{S}$ (recorded as E), and Simpson's Diversity Index, $D = \sum p_i^2$ (recorded as 1 – D; Magurran 2004) at each sample location for each biotic community. We used the averaged community metric data from sampling points along each trail as replicates for comparisons among the parks. We investigated if metric values for each biotic community differed across the parks using a series of one-way ANOVAs ($\alpha = 0.05$ for all statistical analyses; SAS/STAT software Version 9.3) or Kruskal-Wallis tests (R Version 3.1.2.) with Tukey's and Dunn's post hoc tests, respectively. Additionally, we used the Bray-Curtis similarity Index (R Version 3.1.2.) to investigate differences in species composition among parks (Su *et al*. 2004).

Results

We recorded 70 avian species, 65 understory vegetation species, and 83 overstory vegetation species using 2,043 avian point counts and 227 vegetation subplots. Species richness did not differ for avifauna ($F_{3,22} = 0.50$ $P = 0.685$), understory vegetation ($F_{3,22} = 2.85$ $P = 0.060$), or overstory vegetation communities ($F_{3,22} = 1.67$ $P = 0.202$) across the 4 parks (Table 1). Diversity and evenness values for avifauna ($F_{3,22} = 17.8$ $P \leq 0.001$; $F_{3,22} = 9.57$ $P = 0.003$) and understory vegetation communities ($F_{3,22} = 7.38$ $P = 0.001$; $F_{3,22} = 9.41$ $P \leq 0.001$) were lowest at Mount Magazine (Table 1). Overstory vegetation evenness ($F_{3,22} = 0.71$ $P = 0.559$) and diversity values ($F_{3,22} = 1.61$ $P = 0.242$) did not differ among the parks (Table 1).

Table 1. Community metrics (± 1 SD) for avian, understory woody vegetation, and overstory woody vegetation communities in Mount Magazine, Mount Nebo, Petit Jean, and Pinnacle Mountain State Parks, Arkansas, 2015. Within each community metric and taxon, different letters indicate differences among parks ($P < 0.05$).

Taxon and parks	Richness	Evenness	Diversity
Avian			
Mount Magazine	26.0 ± 3.63[a]	0.49 ± 0.08[a]	0.92 ± 0.01[a]
Mount Nebo	29.0 ± 6.94[a]	0.69 ± 0.11[b]	0.95 ± 0.01[b]
Petit Jean	30.0 ± 7.19[a]	0.65 ± 0.06[b]	0.95 ± 0.01[b]
Pinnacle Mountain	29.0 ± 6.14[a]	0.74 ± 0.10[b]	0.95 ± 0.01[b]
Understory vegetation			
Mount Magazine	25.0 ± 4.80[a]	0.10 ± 0.03[a]	0.55 ± 0.16[a]
Mount Nebo	20.0 ± 6.50[a]	0.27 ± 0.10[b]	0.77 ± 0.10[b]
Petit Jean	23.0 ± 3.33[a]	0.27 ± 0.11[b]	0.81 ± 0.08[b]
Pinnacle Mountain	18.0 ± 5.54[a]	0.28 ± 0.14[b]	0.76 ± 0.07[b]
Overstory vegetation			
Mount Magazine	27.0 ± 6.12[a]	0.35 ± 0.14[a]	0.88 ± 0.04[a]
Mount Nebo	23.0 ± 6.56[a]	0.43 ± 0.14[a]	0.89 ± 0.02[a]
Petit Jean	23.0 ± 2.83[a]	0.32 ± 0.14[a]	0.83 ± 0.08[a]
Pinnacle Mountain	21.0 ± 5.13[a]	0.35 ± 0.15[a]	0.84 ± 0.07[a]

Among the 4 parks, avian species composition was most similar between Petit Jean and Pinnacle Mountain state parks (Tables 2 and 3). Understory vegetation species composition was most similar between Mount Nebo and Petit Jean (Tables 2 and 4) and overstory vegetation species composition was most similar between Mount Nebo and Mount Magazine (Tables 2 and 5). Species composition was most dissimilar between Mount Magazine and Pinnacle Mountain State Parks for all biotic communities.

Table 2. Bray-Curtis similarity values (%) for regional avian (A), understory vegetation (UV), and overstory vegetation (OV) species composition in Mount Magazine, Mount Nebo, Petit Jean, and Pinnacle Mountain State Parks, Arkansas, 2015.

Parks	Magazine	Nebo	Petit Jean	Pinnacle
Magazine A, UV, OV		64.8, 36.3, 52.9	53.8, 41.3, 44.2	51.3, 26.4, 41.5
Nebo A, UV, OV	64.8, 36.3, 52.9		77.6, 72.1, 45.7	78.8, 43.2, 52.6
Petit Jean A, UV, OV	53.8, 41.3, 44.2	77.6, 72.1, 45.7		79.5, 50.0, 45.5
Pinnacle A, UV, OV	51.3, 26.4, 41.5	78.8, 43.2, 52.6	79.5, 50.0, 45.5	

Table 3. Point count totals for the 10 most abundant avian species observed in Mount Magazine, Mount Nebo, Petit Jean, and Pinnacle Mountain State Parks, Arkansas, 2015.

Mount Magazine		Mount Nebo		Petit Jean		Pinnacle Mtn.	
Species	Count	Species	Count	Species	Count	Species	Count
Ovenbird	115	Red-Eyed Vireo	77	Red-Eyed Vireo	61	Carolina Wren	51
Indigo Bunting	84	Indigo Bunting	53	Carolina Wren	58	Tufted Titmouse	50
Red-Eyed Vireo	76	Carolina Chickadee	51	Carolina Chickadee	54	Red-Eyed Vireo	48
Black & White Warbler	55	Black & White Warbler	49	Tufted Titmouse	51	Carolina Chickadee	46
Eastern Wood Pewee	42	Northern Cardinal	46	Northern Cardinal	47	Northern Cardinal	44
Carolina Chickadee	35	Carolina Wren	44	Indigo Bunting	46	Pine Warbler	41
Summer Tanager	26	Tufted Titmouse	42	American Crow	44	Indigo Bunting	40
Hooded Warbler	23	Summer Tanager	41	Blue Gray Gnatcatcher	40	Summer Tanager	40
Scarlet Tanager	23	Eastern Wood Pewee	35	Black & White Warbler	37	Blue Jay	39
Blue Jay	22	Blue Gray Gnatcatcher	32	Pine Warbler	32	Blue Gray Gnatcatcher	27

Table 4. Count totals for the 10 most abundant understory woody vegetation species observed in Mount Magazine, Mount Nebo, Petit Jean, and Pinnacle Mountain State Parks, Arkansas, 2015.

Mount Magazine		Mount Nebo		Petit Jean		Pinnacle Mtn.	
Species	Count	Species	Count	Species	Count	Species	Count
Virginia Creeper	8894	Virginia Creeper	1470	Virginia Creeper	1614	Blueberry *spp.*	1070
Blackberry *spp.*	1233	Northern Red Oak	541	Blueberry *spp.*	482	White Oak	767
Blueberry *spp.*	728	White Oak	365	Pignut Hickory	400	Virginia Creeper	359
Northern Red Oak	476	Blackberry *spp.*	233	Northern Red Oak	327	Shortleaf Pine	334
White Oak	391	Blackgum	209	Blackberry *spp.*	320	Blackberry *spp.*	217
Pignut Hickory	316	Flowering Dogwood	205	White Oak	254	Northern Red Oak	168
Black Locust	269	Silver Maple	193	Flowering Dogwood	105	Blackjack Oak	161
Rose *spp.*	226	Pignut Hickory	192	Blackgum	92	Pignut Hickory	139
Sassafras	129	Paw Paw	159	Silver Maple	84	Blackgum	137
Privet *spp.*	123	Blueberry *spp.*	132	American Beautyberry	83	American Beautyberry	91

Table 5. Point count totals for the 10 most abundant overstory woody vegetation species observed in Mount Magazine, Mount Nebo, Petit Jean, and Pinnacle Mountain State Parks, Arkansas, 2015.

Mount Magazine		Mount Nebo		Petit Jean		Pinnacle Mtn.	
Species	Count	Species	Count	Species	Count	Species	Count
Pignut Hickory	732	Pignut Hickory	382	Shortleaf Pine	2053	Shortleaf Pine	375
White Oak	388	Blackgum	261	Pignut Hickory	747	Pignut Hickory	352
Northern Red Oak	369	Eastern Red Cedar	255	Winged Elm	382	Sweet Gum	222
Mockernut Hickory	225	Northern Red Oak	188	Northern Red Oak	299	White Oak	165
Blackgum	168	White Oak	188	American Elm	250	Post Oak	152
Persimmon	153	Paw Paw	148	Sweet Gum	225	American Elm	132
Black Cherry	148	American Elm	129	Eastern Red Cedar	194	Blackgum	91
American Elm	126	Post Oak	125	White Oak	186	Northern Red Oak	83
Downey Serviceberry	126	Flowering Dogwood	117	Downey Serviceberry	185	Mockernut Hickory	81
Sassafras	120	Silver Maple	75	Blackgum	177	Shumard Oak	72

Discussion

We observed no differences in species richness for avian or woody vegetation communities across the 4 parks. Prior research suggests that species richness at the local scale is partly influenced by regional and geological processes such as historic land use, climate, topography, and soil conditions (Harrison *et al*. 2006). Considering that all 4 study sites were mountainous parks of similar latitude and regional habitat condition, the lack of differences in species richness then is unsurprising. Similarities in community richness may have reflected species present that have adapted to the same historical patterns of temperature, precipitation, and topography in west-central Arkansas.

A positive relationship exists between vegetation community structure and avian communities at local scales via the availability of resources and the amount of protective vegetation cover (Böhning-Gaese 1997; Cueto and Casenave 1999; Gill *et al*. 2001; Rahbek and Graves 2001). Given that park management decisions can affect vegetation communities within state parks through vegetation removal and trail upkeep, the lower values of avian and understory vegetation community evenness and diversity we observed at Mount Magazine compared to the other parks could be related to their management practices. For example, daily decisions on trail upkeep, design, and the clearing of debris within state parks can promote unevenness in woody vegetation through the removal of disturbance-intolerant species. To promote recreation in state parks, park managers will alter trail structure and vegetation with respect to the desired purpose of the trail (Marion *et al*. 2011). This may explain why Mount Magazine had some of the lowest levels understory vegetation and avian evenness among the parks. Many trails within Mount Magazine had primarily grassy substrates and led to major tourism structures (i.e., the lodge, visitor center, and picnic areas). Consequently, trails in Mount Magazine were regularly mowed and had branch trimming to allow for greater ease of travel to these structures compared to trails within the other parks that did not lead to major structures of interest. Thus, these modifications to understory woody vegetation communities from recreational use and park management may have led to cascading effects on the surrounding avian communities in Mount Magazine that depend on trailside vegetation for visual cover and resources (Gill *et al*. 2001).

The lack of differences in overstory woody vegetation communities among the 4 parks may also be attributed to park management decisions. State parks often do not allow for major timber removal within park boundaries and typically alter woody vegetation only in conjunction with park management decisions. Overstory woody vegetation communities were also likely influenced by long-term patterns of climate, human land use, and topography within the region.

Similarities in species composition were primarily observed between the 3 parks located within the Arkansas River Valley, likely due to similarities in historic topography and land use among the parks in that ecoregion. Ecoregions are identified based on similarities in abiotic and biotic factors such as soil type, historic land use, and geology (USEPA 2016). Given that Mount Magazine, Mount Nebo, and Petit Jean occurred in the same ecoregion, it was expected that the biotic community compositions would be highly similar. Of the 4 parks, Mount Magazine and Pinnacle Mountain were of greatest geographical distance from each other and existed in 2 different ecoregions. This distance may have translated into differing abiotic pressures acting on park flora and fauna, resulting in the dissimilarities in biotic community composition

between the 2 parks that we observed (USEPA 2016).

Conclusions

Biotic communities within protected areas may respond differently to anthropogenic and natural influences depending on the specific management objectives and habitat structures within each park. We observed no differences in species richness for any of the communities studied. However, there was slight evidence for a possible relationship between avian and understory vegetation evenness and diversity in Mount Magazine, which had the lowest values of both metrics for both communities. These results underscore the importance of researching how local scale changes in park management strategies and habitat structure can influence biotic communities across a landscape. Future research extending the comparisons of biotic communities at a larger scale may benefit protected area managers by providing baseline sets of biotic community data which could then be used to develop holistic management strategies that encompass the collective anthropogenic and environmental effects shaping local state park flora and fauna.

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Phylogeography and Vicariant Separation of Two River Darters, *Percina uranidea* and *Percina vigil,* from rivers that drain the North American Interior Highlands

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Running Title: Ouachita and White River Darter Phylogeography

Abstract

The phylogeography and separation of two river darters, *Percina uranidea* and *P. vigil* were investigated through sequencing of the mitochondrial Cytochrome B and Cytochrome Oxidase genes. These molecular markers revealed the darters exhibit moderate genetic divergence between two large river drainage systems within the Mississippi River basin associated with the Interior Highlands of midwestern North America. An additional haplotype network analysis also supported these trends. Phylogenetic divergence dating indicated that population separation between the river systems occurred after recent Pleistocene glacial events rather than an early Pleistocene separation.

Introduction

The aquatic fauna of the southern United States exhibit a diverse evolutionary and ecological structure due to processes such as regional geology, anthropogenic impacts, climate change and subsequent habitat alterations. In the eastern Gulf Coastal Plain, fish in river systems draining the Appalachian and associated highlands were affected by upland stream changes but also through specific processes more common in lowlands, e.g., river meanders, stream capture, sea level changes, as well as streamflow and sediment load alteration from Pleistocene glacial cycles (Galloway *et al*. 2011; Shen *et al*. 2012 Egge and Hagbo 2015). These and other historical effects created the unique distributions of Gulf Coastal Plain aquatic taxa. In the western Gulf Coastal Plain, consisting primarily of the Mississippi Embayment, river systems evolved through similar processes, but created their own unique faunal distributions (Egge and Hagbo 2015). One of the unique geological features that affected the current streamflow patterns in western Mississippi Embayment was the Interior Highlands.

The Interior Highlands of Arkansas, Missouri, and Oklahoma, USA represent a unique and distinct biogeographic region in North America. The aquatic fauna in the Interior Highlands are often associated with Appalachian and eastern North American connections as many species are derived from eastern species, and the Interior Highlands are considered the western disjunct region of the eastern North American Central Highlands (Mayden 1985; Strange and Burr 1997; Bossu *et al*. 2013). Many of these eastern species exhibit their western North American boundary in the Interior Highlands or near the western periphery of the Highlands (Robison and Buchanan 1988; Trauth *et al*. 2004). The Interior Highlands are separated into the Ouachita and Ozark mountain regions and the Arkansas River Valley, each with a unique geologic structure (Robison and Buchanan 1988; Guccione 1993; The Nature Conservancy 2003; Zollner 2003). The Interior Highlands are considered glacial refugia for many taxa and possess a complex mixture of aquatic fauna. Stream changes precipitated by Pleistocene glaciation cycles resulted in altered species distributions, endemism, relict populations, range expansion, and speciation that have led to the contemporary aquatic fauna (Mayden 1985; Near *et al*. 2001; Near and Keck 2005; Berendzen *et al*. 2010).

Not only have these Pleistocene events caused aquatic species separation between the eastern Highlands and the Interior Highlands, they have affected species distributions within and surrounding the Interior Highlands, i.e., the Ouachita and White Rivers systems. Rivers within the unglaciated Interior Highlands were altered when glacial cycles changed river volumes, lowered sea levels, and allowed stream capture (Mayden 1985, 1988; Elfrink *et al*. 2008; Blanton *et al*. 2013). Several hypotheses highlight events that affected aquatic fauna within and surrounding the Interior Highlands. The Pre-Pleistocene Ouachita River in southern Arkansas may have originated further west and encompassed portions of the present Red River instead of its current origins in the Ouachita Highlands and caused vicariant

population separation in the smaller streams within the pre-Pleistocene Ouachita River (Mayden 1985; Ross 2013). Mayden (1985) proposed that stream drainage alterations throughout the Pleistocene, such as those between the Ouachita and Red River systems, may have caused peripheral isolation and microvicariance in rivers within the Interior Highlands. One large river drainage change includes the extension of the pre-Pleistocene Arkansas River to its larger, current drainage and stream flow pattern, which separated Ozark from Ouachita populations. Another major change occurred when the Pleistocene Mississippi River altered its course multiple times from the eastern edge of the Interior Highlands across the Mississippi Embayment (Mayden 1988; Saucier 1994; Blum *et al*. 2000). These cyclic expansions and reductions in stream volume, flow, and drainage patterns created a unique and complex pattern seen in many aquatic Ozark fauna (Mayden 1988; Hardy *et al*. 2002; Ray *et al*. 2006; Sabatino and Routman 2008; Blanton *et al*. 2013).

Pleistocene glaciation not only affected aquatic populations in Interior Highlands higher gradient, clear streams, they have impacted the fauna in larger streams of the Mississippi Embayment that drain the Interior Highlands. The alteration of drainage patterns in these larger streams such as the formation of the contemporary Mississippi and Arkansas Rivers also changed stream habitat, current flow, and separated populations (Mayden 1985; Ray *et al*. 2006; Lang and Echelle 2011). Understanding the geographic distribution patterns in lowland fish may be further confounded as these populations may have experienced greater connectivity among populations for longer time periods due to the reduced gradient and higher water volumes in these rivers with increased duration of high water events (Lang and Echelle 2011; Egge and Hagbo 2015). Additionally, these populations may have historically experienced greater streamflow stability as larger streams are more likely to persist during drought conditions. These factors may have resulted in larger, more stable fish populations with sufficient genetic variation to slow genetic differentiation among populations. Even with larger population density, these lowland populations were affected by large perturbations such as drainage alterations that occurred during the Pleistocene and isolated populations. To determine Pleistocene effects upon fish species distributions in larger streams and discriminate how vicariance affected fish inhabiting larger river systems draining the Interior Highlands, a phylogeographic analysis was conducted with *Percina uranidea* (Jordan and Gilbert 1887) and *P. vigil* (Hay 1882), two darter species with limited geographic distributions in the larger rivers that drain the Interior Highlands.

Although both species inhabit medium-sized streams that drain the Interior Highlands and inhabit the western Mississippi embayment, *P. uranidea* exhibits a limited distribution when compared to *P. vigil* with the current distribution of *P. uranidea* confined to Arkansas and Missouri. The bulk of *P. uranidea* distribution occurs in Arkansas (Page 1983) with disjunct populations occurring in the White River and Ouachita River drainages. Kuehne and Barbour (1983) reported that *P. uranidea* also occurs in the St. Francis River although the species has not been captured from that river in many years (Robison and Buchanan 1988). Historical records show that the species occurred in, but has since been extirpated from, the lower Wabash River of Indiana and Illinois (Page 1983) and the Ouachita River of Louisiana (Chris Davidson, USFWS, *pers. comm*.). *Percina uranidea* is currently listed as a species of lower risk near threatened (Gimenez 2008), or vulnerable (Arkansas Natural Heritage Commission 2007), and a species of greatest conservation need (Anderson 2006).

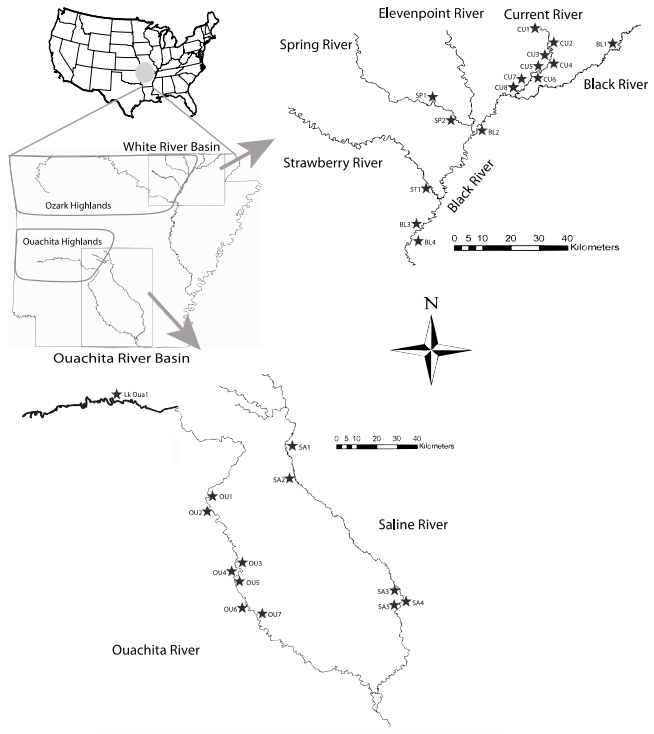

Figure 1. Location map for collected *P. uranidea* and *P. vigil* samples in the White River and Ouachita River basins. See Table S1 for locality information and GenBank Accession data.

Percina vigil, with its greater distribution than *P. uranidea*, ranges from northwest Indiana to southeast Missouri, south to east central Louisiana to northwest Florida. Page (1983) reported that its distribution is sporadic, with locally abundant populations; however, within the state of Arkansas, its distribution mirrors *P. uranidea*, with disjunct populations occurring in the White River and Ouachita River drainages. *Percina vigil* and *P. uranidea* are reportedly found in different habitats within moderate-sized rivers, with *P. vigil* found in shallow habitats with fine gravel or sand bottoms and *P. uranidea* found along gravel bottoms in deeper water, although they are usually syntopic within the state of Arkansas (Robison and Buchanan 1988).

The unique distribution of *P. vigil* and *P. uranidea* in Arkansas can also illuminate if vicariance separation occurred earlier versus later in the Pleistocene. If these fish were affected through the alteration of connections disrupting dispersal during the Pleistocene, the resultant phylogenetic tree would show shorter branches and more haplotype sharing among populations indicating a recent disruption of gene flow among streams. Consequently, molecular clock dating analysis should indicate a more recent divergence between White River and Ouachita River populations. Alternatively, if these populations were separated via early vicariance events, the phylogenetic tree should show a deep separation between the two river drainages with reduced haplotype sharing within river drainages, i.e., a hierarchical haplotype distribution showing reduced haplotype similarity among populations within a river and unique haplotypes among different rivers in a river drainage. The molecular clock dating analysis should also show deep divergence times between river drainages. Furthermore, if early Pleistocene peripheral isolation affected these populations, the phylogenetic tree should exhibit deep branches at the tips with shorter divergence among river basins.

Materials and Methods

Sample collection and preparation

Sixty three *P. uranidea* and 40 *P. vigil* specimens were collected from the Arkansas portions of seven rivers in these darter's historical distribution (28 collection sites): the Black River, Current River, Spring River, Strawberry River, and Eleven Point River of the White River drainage and the Ouachita River and Saline River of the Ouachita River drainage (Figure 1 and S1). Collection sites were based on access ease and favourable sampling conditions, and included an upper, middle, and lower segment of each river within the Arkansas border. The length of the 24 sampled segments measured approximately 123 kilometers.

Fish were sampled with a Missouri trawl (a modified balloon trawl) towed behind a boat at an average depth of 1.76 m (range: 0.46-3.74 m). The net is composed of larger mesh netting (38 mm) encased by smaller mesh netting (6 mm). Compared to other sample methods, the Missouri trawl has been shown to more effectively capture small-bodied, benthic fishes, such as *P. uranidea* and *P. vigil*, in moderate to large river systems (Herzog *et al*. 2005). When sampling conditions were not conducive to using the Missouri trawl (patchy environments, untrawlable stretches due to too much debris, and too shallow water), kick-electrofishing with a backpack shocker and a downstream blocknet was a secondary method to capture darters. Upon capture, the left pectoral and caudal fins were removed and preserved in 100% v/v ethanol in a -20°C freezer. Voucher specimens were deposited in the Arkansas Tech University Fish Collection.

DNA sequencing

Total genomic DNA from fish fin clips was extracted with the FastID genomic DNA extraction kit (GeneticIDNA Inc., USA). Extracted genomic DNA was stored in molecular biology grade water (Sigma Chemical Co., USA) at -20°C until molecular analyses. The entire mitochondrial Cytochrome *b* gene (Cyt *b*) was amplified with primers described in Near *et al.* (2000) and Brogdon *et al*. (2003). In addition, new primers were developed for the mitochondrial Cytochrome Oxidase I (*COX I*) gene through alignment of the gene in three species (*E. radiosum*, GenBank Accession: AY 34348; *P. macrolepida*, DQ 536430; and *I. Furcatus*, AF484165.2): *COX* 1F (Forward primer) 5'- GTG-GCC-ACC-ACA-CGT-TGA-TTC-TTC-TCG -3' and *COX I*-1500R (Reverse Primer) 5'- GCR-GGC-TCT-TCA-AAT-RTR-TGG-TAG-GG -3'. These mitochondrial genes appear to be well suited for delineating intraspecific relationships and may be better suited for this purpose than nuclear genes such as RAG1 and S7 intron (Near *et al*. 2011, Blanton *et al*. 2013). However, mitochondrial introgression is reported in some darters, but does not appear to be as significant in *Percina* species (Near *et al*. 2011).

Each PCR reaction for Cyt *b* and *COX I* was performed in 25-µL aliquots with the following ingredients: 10-µL total genomic DNA (10 – 50 ng),

1X Taq Buffer (150 mM Tris-HCl pH 8.5, 40mM $(NH_4)_2SO_4$, 3.0mM $MgCl_2$. 0.2% v/v Tween 20), 1 mM for each dNTP, 0.5 µM of each primer, 6.25 units REDTaq DNA polymerase (Sigma Chemical Co., USA), 1.6% v/v Dimethyl sulfoxide, 0.6% w/v BSA, and 1.6% v/v Formamide. The cycling conditions consisted of an initial denaturation period of five minutes at 94 °C followed with 30 one-minute cycles of 94 °C, 50 °C annealing, 72 °C extension, and a final seven-minute extension at 72 °C. After PCR products were verified with agarose electrophoresis in a 0.9% w/v agarose concentration, they were GeneCleaned to remove PCR impurities (Bio 101 Inc., USA). Forward and reverse DNA sequencing was performed with PCR primers for both sequences at the UAMS DNA Core Sequencing Facility on an Applied Biosystems 3100 Genetic Analyzer, Big Dye Terminator Chemistry, Kit version 1.1 (Foster City, CA, USA). For *COX I*, two additional internal primers along with the previous PCR primers were employed to provide additional sequencing products for a more complete sequence contig: *COX I*-961F 5'- TTT-AGC-TGA-CTC-GCA-ACY-CTT-C -3' and *COX I*-1185R 5'- GCC-CGA-GAA-TAG-MGG-GAA-TCA-GTG -3'.

After sequencing, all trace files were reviewed by eye and all ambiguous bases removed from further analysis. Alignment of the sequence data was conducted with Clustal X and Geneious Pro 3.7 (Thompson *et al*. 1997; Drummond *et al*. 2009). After the initial alignment and contig creation, all sequences were converted into their amino acid sequences to verify if any internal stop codons existed. All sequences were deposited in GenBank with the following accession numbers for Cyt *b*: KC211117-KC211117. GenBank accession numbers for *COX I* sequences are KC211058-KC211116.

Data Analysis and phylogenetic tree production

Several outgroups from GenBank records were included for the analyses. The Cyt *b* and *COX I* sequences were not concatenated as outgroup sequences retrieved from GenBank were varied in sequence size among individuals and *COX I* sequences were typically smaller than our sequences (650bp vs >1kb). For the Cyt *b* Bayesian analysis, 18 sequences were retrieved from GenBank that included the following outgroups: *P. caprodes, P. macrolepida, P. lenticula, P. antesella, P. copelandi, P. aurora, P. brevicauda, P. tanasi,* and *P. shumardi* (Table S1). The *COX I* outgroups included these species: *P. caprodes, P. maculata, P. lenticula, P. antesella, P. copelandi, P. aurora, P. brevicauda, P. tanasi,* and *P. shumardi* (Table S1). These outgroups were identified through examination of the closest species to *P. uranidea* and *P. vigil* (Near *et al*. 2011). A total of 65 additional *P. uranidea* and *P. vigil* sequences were added for *COX I* from GenBank to include samples from outside Arkansas (Table S1).

All aligned DNA sequences were entered into MODELTEST version 3.7 in HyPhy, and the model of nucleotide sequence evolution (Cyt *b*: GTR+I+G, -lnL = 4342.0; *COX I*: GTR+I+G, -lnL = 4650.9) was chosen with the Akaike (AIC) criteria (Posada and Crandall 1988; Posada 2009; Kosakovsky *et al.* 2006). These sequences were analysed with Bayesian methods with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) with these parameters: four separate Metropolis-coupled Monte Carlo Markov chains, random starting trees with 20 X 10^6 generations with samples taken every 100 generations, and 25% of the resultant trees removed as burn-in. A 50% majority-rule consensus tree was produced with nodal posterior probability support from the four runs post burn-in. The average standard of split frequencies was examined to determine if they dropped to a low, convergent value below 0.005. The outputs from the Bayesian analyses with TRACER v1.6 (Drummond *et al*. 2012) were reviewed to evaluate the robustness of the Bayesian analyses with respect to burn-in, effective sample size, stationary distribution, and posterior.

Population Statistics

As population divergence was considered to be potentially minor, additional analyses were conducted to better understand population structure and evolution. Analyses that consider population level processes such as a multitude of haplotypes in populations and recombination encompass parameters that may not be considered in strict phylogenetic analyses (Clement *et al.* 2000; Althoff and Pellmyr 2002; Hey and Machado 2003). Haplotype network analysis was conducted on Cyt *b* sequences in TCS with 95% connection limits (Clement *et al*. 2000). Any network loops that caused ambiguities were resolved according to Pfenninger and Posada (2002).

To further explore patterns in our data, several population genetics statistics were conducted. These statistics were summarized with Arlequin 3.01 (Excoffier *et al*. 2005). Populations were grouped into two regional groups corresponding to their current disjunct distributions in the Ouachita and White river basins. These statistics were conducted with both *P. uranidea* and *P. vigil* to determine if any evidence of recent expansion and non-neutrality of DNA sequences

existed in these regional groups. To test this hypothesis, Fu's F_s and Tajima's D were calculated in Arlequin 3.01 (Tajima 1989; Fu 1997; Excoffier *et al*. 2005). Significant negative values of these statistics indicate non- neutrality and population expansion: Fu's F_s below a p-value of 0.02 indicate population expansion (Fu 1997; Excoffier *et al*. 2005).

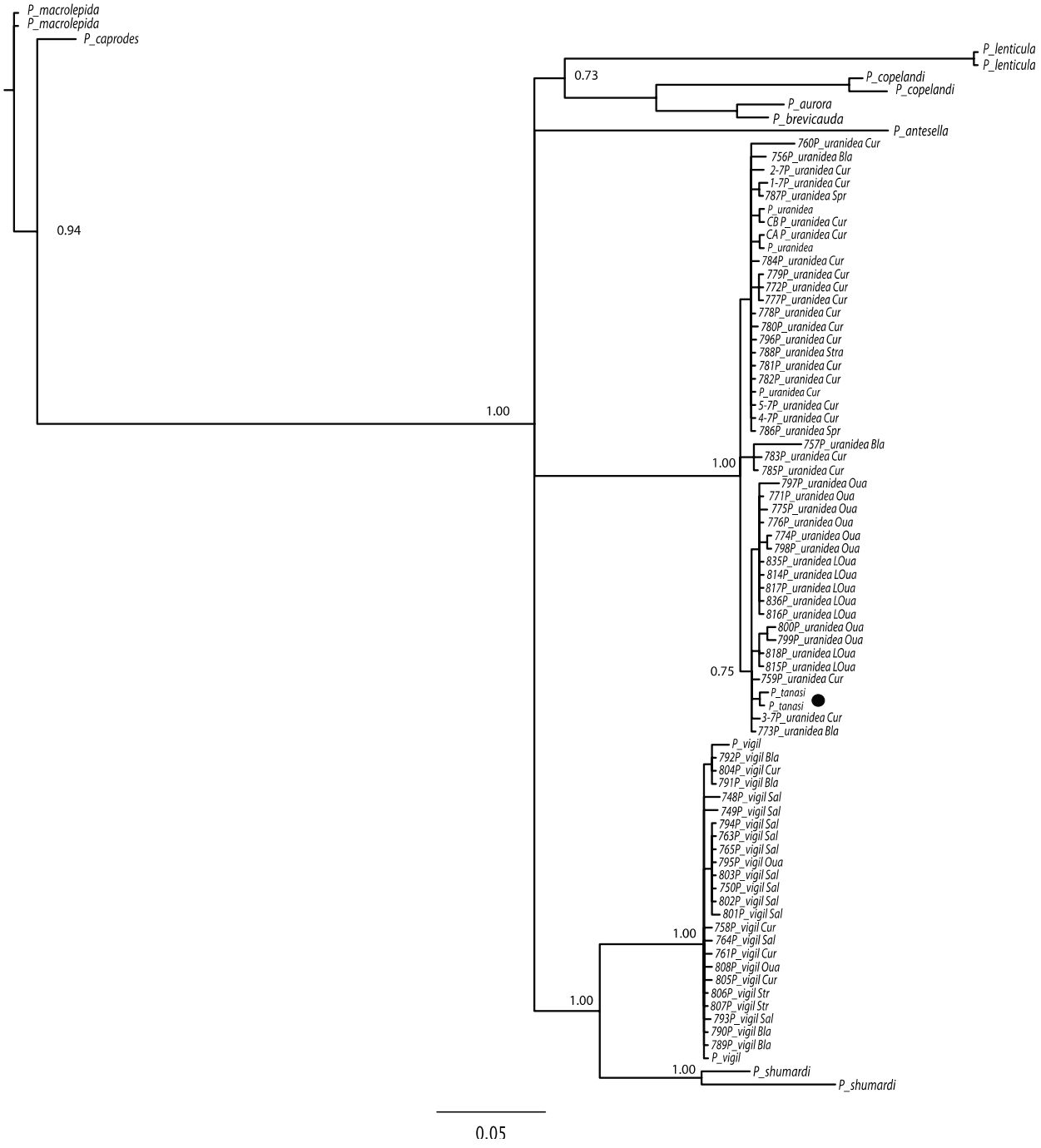

Figure 2. A 50% majority rule consensus phylogram created with Cyt b sequences in Mr Bayes. Clade posterior probabilities are shown at the major nodes. *P. tanasi* sequences are identified with a •. River designations are as follows: Bla = Black river, Cur = Current River, Spr = Spring River, Stra = Strawberry River, Oua = Ouachita River, LOua = Lake Ouachita, and Sal = Saline River

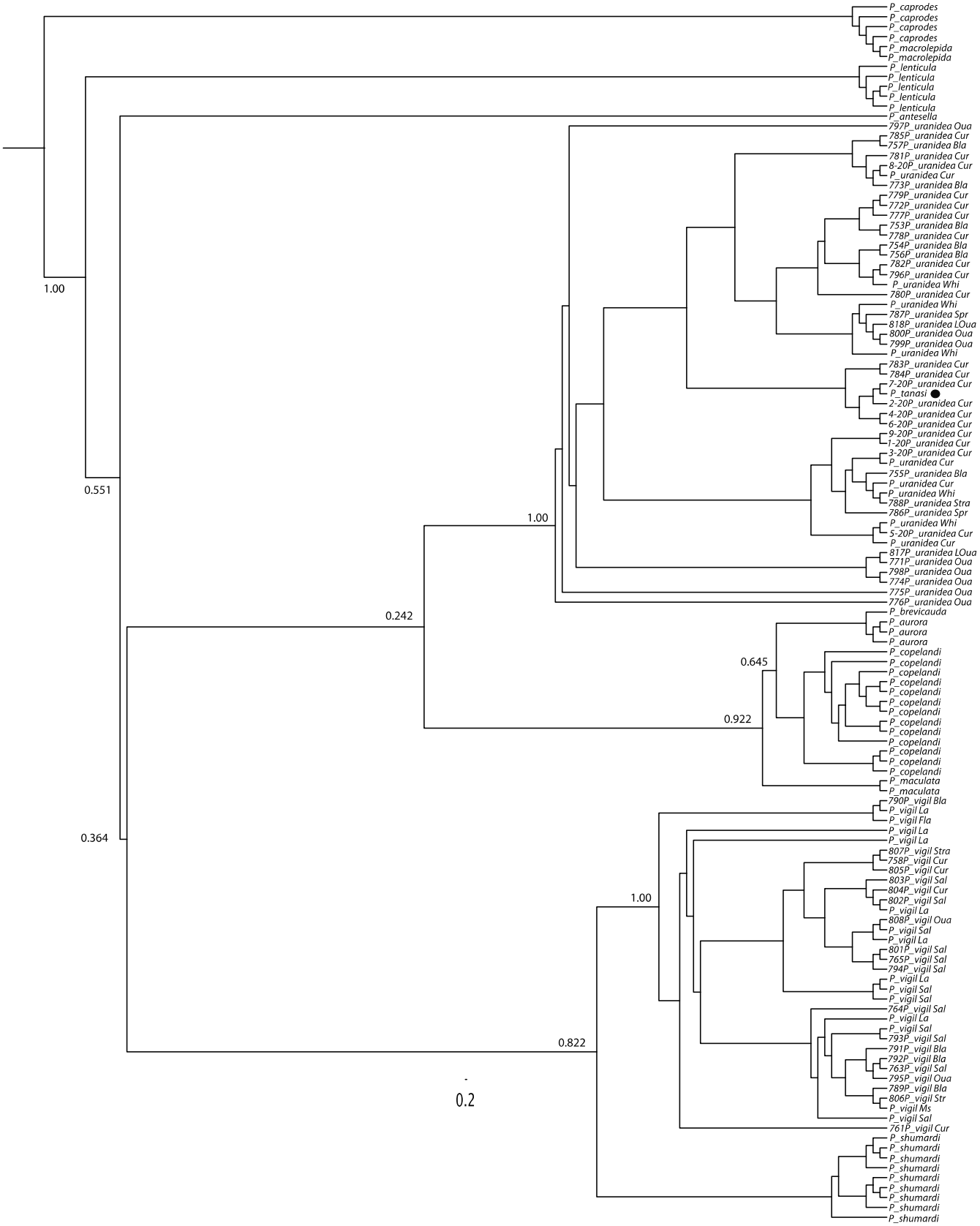

Figure 3. A 50% majority rule consensus phylogram created with COX I sequences in Mr Bayes. Clade posterior probabilities are shown at the major nodes. *P. tanasi* sequences are identified with a •. River designations are as in Figure 2.

Divergence Dating

To further investigate migration and date population separation, Cyt *b* coalescent analyses were conducted with *BEAST v.2.1.2. This analysis estimates several parameters (phylogeny & divergence dates) using a relaxed clock model (Kumar *et al*. 2009; Drummond *et al*. 2012; Bouckaert *et al*. 2014). Dating analysis was not conducted with *COX I* as outgroup sequences length were significantly shorter than those produced in this study. The divergence date estimates with the BEAST software were produced with similar parameters to Bayesian analysis done in MrBayes but increased generation time ($100X10^6$ generations & 20% burn-in). The clock model was calibrated with the proposed Arkansas River expansion in the Sangamon ~ 0.105 mybp (Mayden 1985; Elfrink 2007; Tripsanas *et al.* 2007). The Cyt *b* estimated pairwise rate of nucleotide substitution was set to 1.80% per myr as reported in Near *et al*. (2011). In these analyses, *P. uranidea* was constrained from *P. vigil*, then a further constraint was done within these species to reflect separation into Ouachita River and White River drainages. These constraints were conducted with a normal distribution in the nodes with a calibrated Yule model prior. All Bayesian outputs produced through BEAST were also reviewed in TRACER for robustness in a similar manner to the Mr Bayes simulations. The resultant trees were summarized in TreeAnnotator v1.6.1 to create a 50% majority-rule consensus maximum clade credibility tree.

Results

Data Analysis and phylogenic tree production

For Cyt *b*, 65 samples of 1190bp were sequenced and an additional 18 sequences were added from GenBank. The mean base composition of the sequenced samples was A = 0.22, C = 0.32, G = 0.17, and T = 0.28 with 309 variable sites. For Cyt *b*, 36 haplotypes were recovered for *P. uranidea* and 19 for *P. vigil*. The 58 individuals sequenced for *COX I* produced contigs of 1495bp with 261 variable sites and a mean base composition of A = 0.24, C = 0.29, G = 0.19, and T = 0.29, with an additional 65 sequences of 670bp included from GenBank to improve the phylogenetic analysis with this locus. For COX1, 39 haplotypes were recovered for *P. uranidea* and 26 for *P. vigil*.

Both Bayesian trees mirrored the trees for these species reported in Near *et al*. (2011) with reciprocal monophyly in both *P. uranidea* and *P. vigil* and high posterior probabilities (Figures 2 and 3). The Cyt *b* tree was characterized with a harmonic mean –ln*L* value of 4676.33 and the *COX I* tree exhibited a harmonic mean –ln*L* value of 5129.55. The Cyt *b* tree did not resolve relationships among *Percina* taxa as well as the *COX I* tree, but exhibited higher posterior probabilities for all nodes when compared to the *COX I* tree. Furthermore, both the *Cyt b* and *COX I* trees suggest *P. uranidea* is paraphyletic with *P. tanasi* nestled within this clade, and neither tree reveals a distinct structuring of White River and Ouachita River drainages as haplotypes were not exclusive to a drainage nor river, i.e., each tree showed a mixture of Ouachita and White River individuals without clearly separating the two drainages.

Population and gene diversity statistics, with divergence dating

The Cyt *b* haplotype network analysis created three unconnected networks at the 95% connection limit. These networks consisted of two *P. uranidea* and one *P. vigil* (Figure 4). All networks mimic the phylogenetic analyses with only one network showing a clear separation between White River and Ouachita River drainages. The genetic diversity statistics summarized in Arlequin suggests population expansion took place for both species in the two river drainages for all values of Fu's Fs except for *P. uranidea* in the Ouachita River drainage (Table 1).

The divergence dating analysis under a coalescent expansion growth prior created a tree with a likelihood of -4185.49 (Figure 5). The dates calculated for the separation of White River drainage populations from Ouachita River drainage populations correlates with a Sangamon divergence date for both species (*P.uranidea*, 0.0997 mybp and *P. vigil*, 0.1326 mybp).

Discussion

The limited divergence between the White River and Ouachita River drainage populations suggests these populations were connected until recently or populations retained substantial genetic variation due to slow allele loss after a separation event (Figure 4 and 5). Based upon the ecological characteristics of both species, it is likely that the contemporary streamflow patterns of the large rivers, such as the Arkansas and Mississippi, altered habitat requirements with gradual local extinctions to create the distributions observed in *P. uranidea* and *P. vigil*. As both species exist in deeper waters of medium-sized rivers, populations likely contained densities that prevented

bottlenecks and random effects due to genetic drift. As the data also suggest population expansion (Table 1), some populations acted as source populations from which recolonization could occur to nearby habitats, newly created as these complex and dynamic Gulf Coastal Plain river systems evolved to their modern distributions and stream characteristics.

The *COX I* tree and the haplotype network (Figure 2 and 4) shows *P. uranidea* with greater haplotype segregation in the White River as compared to *P. vigil*. These results suggest specific habitat requirement differences between the species may not only affect the geographic distribution of the species, but may also affect gene flow among populations. *Percina uranidea's* preference for deeper waters and gravel bottoms may promote more isolation among populations and limit gene flow.

Limited genetic divergence is also present in the Ouachita River drainage as *P. uranidea* populations in the mid Ouachita River (below Lake Catherine) possess different haplotypes from those in the upper Ouachita River (Lake Ouachita population - *LOua*), yet exhibit low divergence from mid Ouachita River and do not form a separate clade in either the Cyt *b* or *COX I* trees (Figure 2 and 3). However, the 11step separation in haplotype network B (Figure 4) are the *LOua* population samples and shows gene flow disruption via river impoundment affects population genetic structure. The upper Ouachita population is approximately 150 km upstream from the mid Ouachita population and also separated by three large reservoirs. *Percina vigil* also reflects the limited *COX I* divergence in the Ouachita River drainage as sequences from Louisiana, Mississippi, and Florida are mixed with those in the Saline and even those in the White River drainage (Figure 3).

Table 1. River drainage diversity indices for *P. uranidea* and *P. vigil* in *Cyt b* and *COX 1* sequences.

River Drainages	Sample & Haplotype #'s ( )	Gene Diversity ± SE	Nucleotide Diversity ± SE	Fu's Fs	Tajima's D
P. uranidea					
Cyt b White River	30 (16)	0.862 ±0.0579	0.0033 ±0.0019	-6.345 p=0.002	-2.00 p=0.006
Ouachita River	15 (7)	0.781 ±0.1020	0.0019 ±0.0012	-1.685 p=0.134	-1.17 p=0.132
COX 1 White River	36 (30)	0.984 ±0.0125	0.0047 ±0.0028	-26.28 p=0.000	-2.02 p=0.006
Ouachita River	9 (9)	1.000 ±0.0520	0.0036 ±0.0022	-4.843 p=0.003	-0.09 p=0.519
P. vigil					
Cyt b White River	13 (10)	0.949 ±0.0500	0.0016 ±0.0011	-7.687 p=0.000	-1.71 p=0.035
Ouachita River	12 (9)	0.939 ±0.0580	0.0011 ±0.0008	-7.817 p=0.000	-1.28 p=0.115
COX 1 White River	10 (10)	1.000 ±0.0450	0.0029 ±0.0018	-6.650 p=0.000	-0.67 p=0.272
Ouachita River	24 (16)	0.917 ±0.0482	0.0028 ±0.0019	-15.533 p=0.000	-2.085 p=0.003

With regards to the dispersal or vicariance models, a recent vicariance event is supported rather than a pre-glaciation vicariance event as neither the phylogenetic analyses nor the haplotype network analysis show a haplotype distribution consistent with dispersal from a refugia; i.e., basal haplotype populations with derived haplotype populations that reflect expansion (Berendzen *et al*. 2008; Blanton *et al*. 2012). In addition, the divergence dating analysis suggests a Pleistocene division between the two river basins (Figure 5). However, the *COX I* tree shows that the most basal *P. uranidea* haplotypes are those from the Ouachita River drainage, which may suggest a longer divergence period in this region.

The phylogenetic data also suggests a paraphyletic relationship in *P. uranidea* as *P. tanasi* is nested within *P. uranidea* haplotypes in both Cyt *b* and *COX I* trees. This paraphyletic relationship in these darters is not reported in other *Percina* phylogenetic studies (Near *et al*. 2011) and may represent incomplete lineage sorting in specific populations of these darters. As *Percina* darters inhabit deeper waters of streams and rivers, which may house larger populations, population divergence, speciation, and lineage sorting may require longer divergence periods. Conversely, this relationship could be an artifact of mitochondrial introgression into *P. tanasi*. If mitochondrial introgression has occurred, this relationship would provide evidence of a second example in the *Percina* genus (Near *et al*. 2011). As nuclear genes were not sampled, a definitive conclusion regarding introgression is premature.

In conclusion, our results provide support that the Pleistocene Arkansas River expansion created a substantial barrier, reducing gene flow between the Ouachita and White River systems. In addition, our results suggest that further examples of incomplete lineage sorting may exist in other darter species and may lay hidden within unsampled haplotypes further complicating the phylogenetic resolution of species within this genus. Due to the complexity of darter phylogenetics, it appears to be fruitful to conduct further extensive population level sampling within species in the genus *Percina* to better illustrate the extent and complexity of speciation within this genus.

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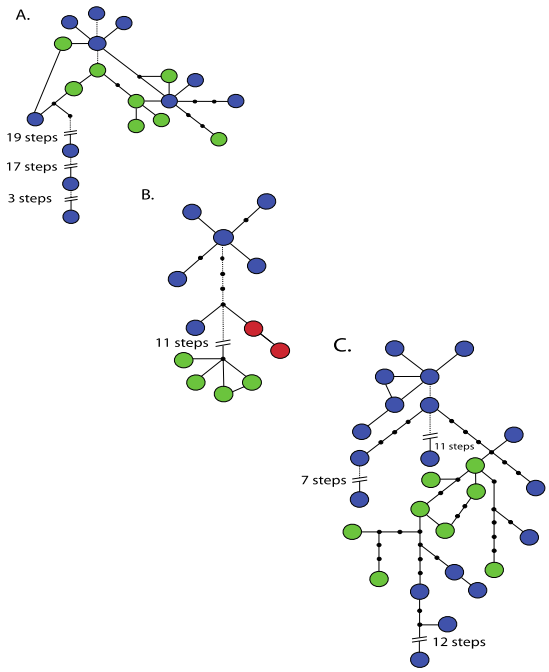

Figure 4. Haplotype networks for Cyt b created in TCS. Blue circles represent White River basin haplotypes, green circles represent Ouachita River haplotypes, and red circles represent *P. tanasi*. *P. uranidea* haplotypes are shown in network A & B: *P. vigil* in network C.

Supporting Information

Table S1. Individual sample information with GenBank Acession numbers. Taxa accessed through GenBank follow DNA sequences created in this study.

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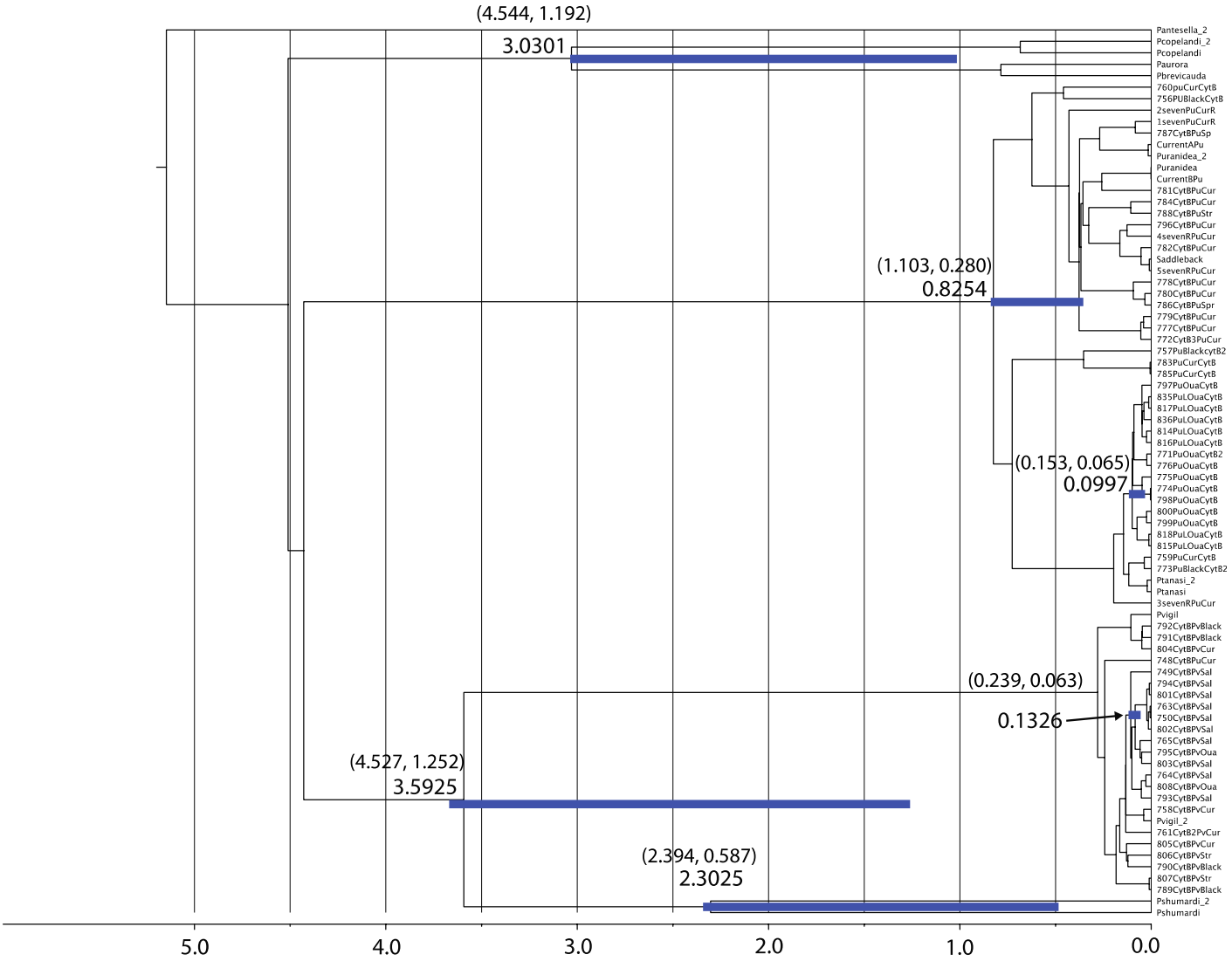

Figure 5. A 50% majority-rule consensus maximum clade credibility tree showing estimated divergence estimates created through a relaxed clock model in BEAST* with Cyt b sequences. *P. uranidea* and *P. vigil* were constrained into Ouachita River and White River populations. Mean clade ages are shown at the nodes with 95% uncertainty lower bound ranges shown in blue and 95% uncertainty ranges shown in parentheses. The scale represents age estimates in mybp.

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Coccidian Parasites (Apicomplexa: Eimeriidae) of Arkansas Herpetofauna: A Summary with Two New State Records

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Running Title: Coccidians of Arkansas Herpetofauna

Abstract

Coccidian parasites (Protista: Apicomplexa: Eimeriidae) commonly infect reptiles, and to a lesser degree, amphibians. The family Eimeriidae includes at least 18 genera and 3 of them, *Caryospora*, *Eimeria*, and *Isospora* have been reported previously from various Arkansas herpetofauna. Over the past 3 decades, our community collaborative effort has provided a great deal of information on these parasites found in amphibians and reptiles of Arkansas. Here, we provide a summary of all coccidians reported from herptiles of the state as well as provide 2 new state records for coccidians from non-native Mediterranean geckos, *Hemidactylus turcicus*.

Introduction

Coccidians (Eimeriidae) are endoparasites that belong to the protist phylum Apicomplexa, suborder Eimeriorina. They are some of the most ubiquitous of all taxa of protists found in vertebrate animals. However, except for some that are medically or of veterinary importance in domestic animals and humans, they are most likely the least studied and understood of all vertebrate endoparasites.

In general, coccidians have a rather complex life cycle (Fig. 1), with 3 sequential stages, including reproduction by endogenous (intracellular) merogony and gametogony followed by sporogony, which is extracellular (in the form of the oocyst). The oocyst represents the cyst containing the fertilized cell (zygote). Interestingly, the oocyst is highly resistant to all known fixative techniques, and the majority of all species descriptions (diagnoses) are based on the sporulated oocyst. No satisfactory methods are known to preserve the structural integrity of the oocyst

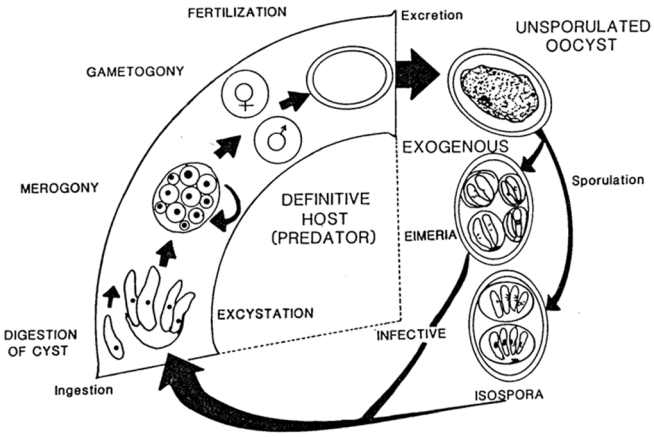

Figure 1. Life cycle of *Eimeria* and *Isospora* spp. (Redrawn from Fayer 1982).

permanently, so the taxonomy of coccidians has generally been non-specimen based. As a result, many species are described solely on measurements of morphological structures of the infective sporulated oocyst (Fig. 2), some additional key qualitative features (particularly shape), line drawings, photomicrographs, and consideration of host species and geographic range. Only within the last couple of decades have molecular techniques (amplifying DNA) been applied to coccidians to help supplement morphological data (see Morrison *et al*. 2004; Jirků *et al*. 2002, 2009; Megía-Palma *et al.* 2015).

There are distinct morphological and endogenous developmental differences in 5 of the genera of coccidians that occur in amphibians and reptiles in the state. The genus *Eimeria* Schneider, 1875 is the largest genus in the family with oocysts having 4 sporocysts, each with 2 sporozoites; *Isospora* Schneider, 1881 has oocysts with 2 sporocysts, each with 4 sporozoites; *Caryospora* Léger, 1904 has oocysts possessing a single

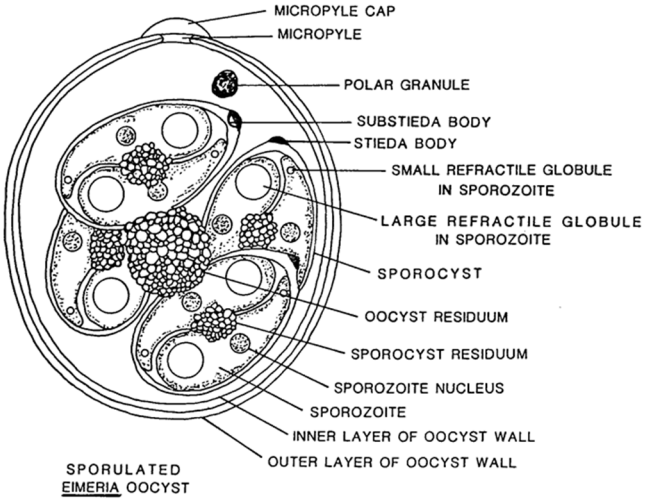

Figure 2. Sporulated *Eimeria* sp. oocyst showing morphological structures used to distinguish species. (Redrawn from Levine 1978).

sporocyst, each with 8 sporozoites; in addition, the typical life-cycle of a caryosporan species has both direct and facultatively heteroxenous life-cycle components (see Duszynski and Upton 2009); *Acroeimeria* Paperna and Landsberg, 1989 have oocysts that are small, spheroidal, and shed in the unsporulated condition, and, when sporulated, they are similar to those of *Eimeria* species, to which they are closely related; also, they have endogenous development with a parasitophorous vacuole that begins to bulge above the surface of the intestinal mucosal cells as meronts and gamonts continue to grow; the host cell cytoplasm expands as the parasite grows, giving rise to a short, stalk-like structure forming a layer on the surface of the gut mucosa; this endogenous development occurs above the host cell nucleus and below the brush border in the enterocytes of the ileum; lastly, the genus *Choleoeimeria* Paperna and Landsberg, 1989 is restricted to coccidians infecting the gallbladder and biliary epithelium of reptiles, and possesses elongate-ellipsoidal oocysts (L/W ratio >1.5) with 4 sporocysts, each with 2 sporozoites; it is further characterized by sporocysts without a Stieda/substieda body complex, but with longitudinal sutures in their walls.

Among Arkansas herpetofauna, there are several reports of coccidians in reptiles, including those in turtles (McAllister *et al*. 1994a; Duszynski and Morrow 2014), lizards (McAllister *et al*. 1994b), and snakes (Duszynski and Upton 2009). On the other hand, there are fewer reports of coccidia in amphibians (Upton and McAllister 1988; McAllister *et al*. 1993, 2002; Upton *et al*. 1993; Duszynski *et al*. 2007).

In Arkansas, to our knowledge, there were no previous reports of coccidians infecting amphibians or reptiles prior to 1975, when Leon W. Bone, then of the University of Arkansas, reported *Eimeria pseudemydis* Lainson from a red-eared slider, *Trachemys scripta elegans* from Lonoke County (Bone 1975). Since then, there has been an explosion of reports describing new and previously described coccidians of Arkansas herpetofauna (see citations in McAllister *et al*. 1994; Duszynski *et al*. 2007; Duszynski and Upton 2009; Duszynski and Morrow 2014) but a summary of those species in the state has never been published. Here, we provide a summary of the coccidian parasites within the largest family of the phylum (Eimeriidae) in the amphibians and reptiles of the state as well as document 2 coccidians from Arkansas for the first time.

Materials and Methods

A thorough examination of the published literature was conducted on coccidians previously reported from amphibians and reptiles of Arkansas. In addition, 3 adult Mediterranean geckos (*Hemidactylus turcicus*) were collected in October 2013 and April 2014 from El Dorado, Union County (n = 2), and one in April 2017 from Forrest City, St. Francis County (McAllister and Robison 2017). In addition, a single prairie kingsnake (*Lampropeltis calligaster calligaster*) was found dead on the road in October 2016 in Saline County; all were examined for coccidia. Fresh fecal samples were placed in individual vials containing 2.5% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$). Samples were examined for coccidia using an Olympus BX53 light microscope after flotation in Sheather's sugar solution (specific gravity = 1.30). Measurements were taken on 15 sporulated oocysts using Olympus© cellSens 1.14 digital software and reported in micrometers as means; photographs were taken using Nomarski interference-contrast optics. Oocysts were 780–960 days old when measured and photographed. For light microscopy, tissue samples from the intestine and gall bladder of *H. turcicus* were fixed in 10% neutral–buffered formalin and processed as histological sections following standard methods of staining with hematoxylin and eosin or Pollak trichrome stain (Presnell and Schreibman 1997). A host photovouchers was accessioned into the Arkansas State University Museum of Zoology (ASUMZ) Herpetological Collection, State University, AR as ASUMZ 33619. Photosyntypes of sporulated oocysts were accessioned into the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, NE.

Results and Discussion

Of the amphibian hosts, there are only 5 species of valid coccidians known from the state whereas there are 50 valid species in the reptiles of Arkansas. Indeed, Upton *et al*. (1993) examined 238 caudate amphibians from Arkansas within 7 families and found only 9 (4%) harbored coccidians. Upton and McAllister (1988) also reported low prevalence in 4 anuran amphibians from the state. Concerning reptilian hosts, there are 50 taxa, 18 species in turtles, 8 species in lizards, and 24 coccidian species in snakes.

All 4 of the *H. turcicus* were found to be infected with coccidia as follows: 3 harbored *Acroeimeria lineri* and one was infected with *Choleoeimeria turcicus*. The single *L. c. calligaster* was found to be passing *Caryospora lampropeltis*. Data for each is listed below in the annotated checklist as well as those known from other Arkansas herpetofauna.

Annotated Checklist of Coccidians from Arkansas Herpetofauna

AMPHIBIA: CAUDATA: AMBYSTOMATIDAE
FAMILY EIMERIIDAE MINCHIN, 1903
GENUS *EIMERIA* SCHNEIDER, 1875
***Eimeria opacum* Upton, McAllister and Trauth, 1993**
Host: Marbled salamander, *Ambystoma opacum.*
Locality: Grant Co.
Prevalence: 1/3 (33%).

PLETHODONTIDAE
Eimeria* sp. of McAllister, Upton, and Trauth, 2002, *incertae sedis
Host: Kiamichi slimy salamander, *Plethodon kiamichi.*
Locality: Polk Co.
Prevalence: 1/16 (6%).
Remarks: McAllister *et al*. (2002) found a single *P. kiamichi* passing oocysts they identified as an *Eimeria* species, but did not describe or name it. Duszynski *et al*. (2007) considered this eimerian an *incertae sedis*. Additional samples are needed to determine its identity. The host is a Species of Special Concern in the state.

GENUS *ISOSPORA* SCHNEIDER, 1881
***Isospora hightoni* Upton, McAllister, and Trauth, 1993**
Host: Western slimy salamander, *Plethodon albagula.*
Localities: Grant (type), Independence, Lawrence, Montgomery, Perry, and Pope cos.
Prevalence: 1/6 (33%), 1/1 (100%), 1/2 (50%), 2/5 (40%), 1/2 (50%), and 2/2 (100%), respectively.
Remarks: Among the *ca.* 7,696 species of worldwide amphibians, there are only 11 valid species of *Isospora* (Duszynski *et al.* 2007).

ANURA: HYLIDAE
***Isospora delicatus* Upton and McAllister, 1988**
Host: Illinois chorus frog, *Pseudacris illinoensis.*
Locality: Clay Co.
Prevalence: 1/8 (13%).
Remarks: This frog is found only in Clay County in far northeastern Arkansas (Trauth *et al*. 2004) and is a Species of Greatest Conservation Need in the state (Anonymous 2016).

RANIDAE
***Eimeria fitchi* McAllister, Upton, Trauth, and Bursey, 1995**
Host: Wood frog, *Rana* (=*Lithobates*) *sylvaticus*.
Locality: Izard Co.
Prevalence: 11/13 (85%).
Remarks: This was the first ranid frog in the U.S. documented to harbor coccidia and the host is a Species of Special Concern in the state.

***Eimeria menaensis* McAllister, Seville, Bursey, Trauth, Connior, and Robison, 2014**
Host: Green frog, *Rana* (=*L*.) *clamitans.*
Locality: Polk Co.
Prevalence: 1/20 (5%).

REPTILIA: TESTUDINES: CHELYDRIDAE
***Eimeria chelydrae* Ernst, Stewart, Sampson, and Fincher, 1969**
Host: Common snapping turtle, *Chelydra serpentina.*
Localities: Benton, Boone, and Woodruff cos.
Prevalence: 1/1 (100%) in each co.
Remarks: Oocysts of *E. chelydrae* wrinkle easily in Sheather's sugar solution so it is recommended that the concentrated sugar solution be diluted 50:50 in distilled water when examining oocysts from *C. serpentina* (see McAllister and Hnida 2016).

***Eimeria filamentifera* Wacha and Christiansen, 1979**
Host: *C. serpentina.*
Locality: Boone Co.
Prevalence: 1/1 (100%).

***Eimeria harlani* Upton, McAllister, and Trauth, 1992**
Host: Alligator snapping turtle, *Macrochelys temminckii.*
Locality: Jackson Co.
Prevalence: 1/1 (100%).

Remarks: This is the only coccidian known to date from *M. temminckii*. The host is a Species of Special Concern in the state.

***Eimeria serpentina* McAllister, Upton, and Trauth, 1990**
Host: *C. serpentina*.
Localities: Boone (type) and Carroll cos.
Prevalence: 1/1 (100%) in both counties.

***Isospora chelydrae* McAllister, Upton, and Trauth, 1990**
Host: *C. serpentina*.
Localities: Benton 1/1 (100%), Carroll (type), and Woodruff cos.
Prevalence: 1/1 (100%) in each county.
Remarks: This is only the 4th isosporan known from *ca*. 350 species of turtles worldwide (Duszynski and Morrow 2014; Hnida 2015).

EMYDIDAE
***Eimeria carri* Ernst and Forrester, 1973**
Host: Three-toed box turtle, *Terrapene mexicana* (=*carolina*) *triunguis*.
Locality: Garland, Pope, and Sharp cos.
Prevalence: 3/9 (33%) overall.

***Eimeria chrysemydis* Deeds and Jahn, 1939**
Host: Common map turtle, *Graptemys geographica*.
Locality: Fulton Co.
Prevalence: 1/7 (14%).

***Eimeria doddi* McAllister, Motriuk-Smith, Kerr, Carmen, Seville, and Connior, 2017**
Host: Ornate box turtle, *Terrapene ornata*.
Locality: Benton Co.
Prevalence: 1/3 (33%).
Remarks: The host is a Species of Special Concern in the state.

***Eimeria graptemydos* Wacha and Christiansen, 1976**
Hosts: Southern painted turtle, *Chrysemys picta*, Mississippi map turtle, *Graptemys pseudogeographica kohnii*, *G. geographica*, and Mississippi mud turtle, *Kinosternon subrubrum hippocrepis*.
Localities: Arkansas (see Duszynski and Morrow 2014).
Prevalence: 1/1 (100%), 1/4 (25%), 1/7 (14%), and 2/6 (33%), respectively.

***Eimeria marginata* (Deeds and Jahn, 1939) Pellérdy, 1974**
Hosts: *C. picta*, *G. geographica*, and eastern river cooter, Missouri River cooter, *Pseudemys concinna metteri*.
Localities: Cross and Fulton cos.
Prevalence: 1/1 (100%), 1/7 (14%), and 1/4 (20%), respectively.

***Eimeria mitraria* (Laveran and Mesnil, 1902) Doflein, 1909**
Hosts: *T. m. triunguis*.
Locality: Pope Co.
Prevalence: 1/9 (11%).

***Eimeria ornata* McAllister and Upton, 1989**
Host: *T. m. triunguis*.
Localities: Fulton, Pike, and Union cos.
Prevalence: 9/24 (38%) overall.
Remarks: McAllister *et al*. (2015) documented this coccidian from *T. m. triunguis* and Arkansas, for the first time.

***Eimeria pseudemydis* Lainson, 1968**
Host: Red-eared slider, *Trachemys scripta elegans*.
Locality: Lonoke Co.
Prevalence: 1/1 (100%).

***Eimeria pseudogeographica* Wacha and Christiansen, 1976**
Host: Ouachita map turtle, *Graptemys ouachitensis*.
Locality: Fulton Co.
Prevalence: 1/3 (33%).
Remarks: Bone's (1975) report of *E. pseudemydis* is apparently the first documenting a coccidian in any wild host from Arkansas.

***Eimeria somervellensis* McAllister and Upton, 1992**
Host: *P. c. metteri*.
Locality: Fulton Co.
Prevalence: 2/6 (33%).

***Eimeria tetradacrutata* Wacha and Christiansen, 1976**
Host: *G. geographica*.
Locality: Baxter Co.
Prevalence: 3/7 (43%).

TRIONYCHIDAE
***Eimeria apalone* McAllister, Upton, and McCaskill, 1990**
Host: Western spiny softshell, *Apalone spinifera hartwegi*.
Locality: Conway Co.
Prevalence: 1/3 (33%).

KINOSTERNIDAE

***Eimeria lutotestudinis* Wacha and Christiansen, 1976**

Host: *K. s. hippocrepis*.
Locality: Columbia Co.
Prevalence: 1/6 (17%)

SAURIA: GEKKONIDAE

GENUS *ACROEIMERIA* PAPERNA AND LANDSBERG, 1989

***Acroeimeria lineri* (McAllister, Upton, and Freed, 1988) Paperna and Landsberg, 1989**

Host: *H. turcicus*.
Localities: St. Francis and Union cos.
Prevalence: 1/1 (100%), 2/3 (67%).
Morphology/measurements: Ellipsoidal smooth-walled oocysts (Fig. 3A) were (L × W) 24.1 × 18.1, L/W ratio = 1.3; a polar granule was present but a micropyle and oocyst residuum were absent. Subspheroidal sporocysts measured 7.4 × 6.9, L/W ratio = 1.1; Stieda and substieda bodies were absent but a sporocyst residuum was present, composed of numerous granules in a spheroidal or ovoidal mass.
Site of infection: Intestinal epithelium (Fig. 4 A-B).
Remarks: These measurements are similar to those previously reported for *A. lineri* (McAllister *et al*. 1988) from *H. turcicus* in Louisiana and Texas. We document a new geographic record for *A. lineri*. This coccidian (HWML 139319) has now been reported from non-native populations of *H. turcicus* in Arkansas, Louisiana, and Texas, and native populations in Israel (Paperna and Landsberg 1989).

GENUS *CHOLEOEIMERIA* PAPERNA AND LANDSBERG, 1989

***Choleoeimeria turcicus* (Upton, McAllister, and Freed, 1988) Paperna and Landsberg, 1989**

Host: *H. turcicus*.
Locality: Union Co.
Prevalence: 1/3 (33%).
Morphology/measurements: Elongate to cylindroidal smooth-walled oocysts (Fig. 3B) were (L × W) 35.6 × 17.8, L/W ratio = 2.0; a polar granule was present but a micropyle and oocyst residuum were absent. Ovoidal sporocysts measured 9.7 × 8.2, L/W ratio = 1.2; Stieda and substieda bodies were absent but a sporocyst residuum was present, composed of a compact mass of granules of various sizes.
Site of infection: Gallbladder epithelium (Fig. 4C-D).
Remarks: These measurements are similar to those in the original description previously reported for *C.* (=*Eimeria*) *turcicus* (Upton *et al*. 1988) collected from *H. turcicus* in Texas. We document a new geographic record for *C. turcicus*. This coccidian (HWML 139320) has now been reported from non-native populations of *H. turcicus* from Arkansas and Texas, and native populations from Israel (Paperna and Landsberg 1989) and Egypt (Abdel-Haleem *et al*. 2016).

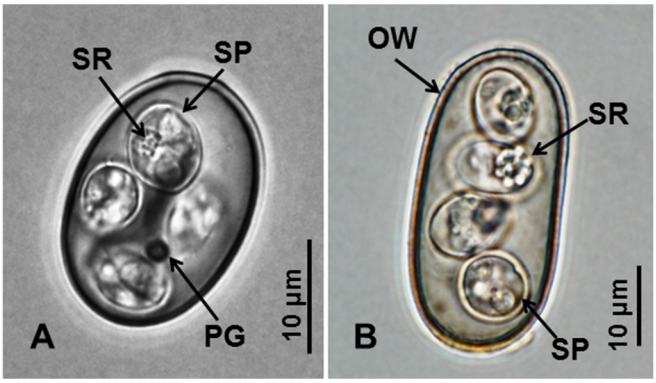

Figure 3. Coccidians from *Hemidactylus turcicus* (Union Co.). A. Sporulated oocyst of *Acroeimeria lineri*. B. Sporulated oocyst of *Choleoeimeria turcicus*. Abbreviations: OW (oocyst wall); PG (polar granule); SP (sporocyst); SR (sporocyst residuum).

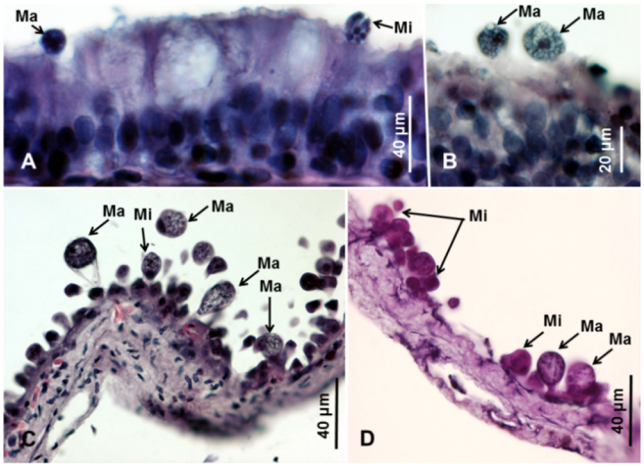

Figure 4. Endogenous stages of *Acroeimeria lineri* and *Choleoeimeria turcicus* in *Hemidactylus turcicus* (Union Co.). A-B. Multinucleate microgamont (Mi) and Macrogamonts (Ma) of *A. lineri* in intestine of *H. turcicus*. C-D. Microgamonts (Mi) and macrogamonts (Ma) in gall bladder epithelium of *C. turcicus* in *H. turcicus*.

POLYCHROTIDAE

***Eimeria robisoni* McAllister, Seville, and Connior, 2014**

Host: Green anole, *Anolis carolinensis*.
Locality: Union Co.
Prevalence: 1/11 (9%).

SCINCIDAE

***Choleoeimeria* (=*Eimeria*) *fasciatus* Upton, McAllister, and Trauth, 1991**

Host: Five-lined skink, *Plestiodon fasciatus*.
Localities: Pope, Washington, and Woodruff cos.
Prevalence: 1/1 (100%), 1/1 (100%), and 1/5 (20%), respectively.
Remarks: This coccidian was originally placed in the genus *Eimeria*; however, developmental stages were clearly shown in gall bladder epithelium (Fig. 5 of Upton *et al.* 1991). Paperna and Landsberg (1989) erected the genus *Choleoeimeria* for eimeriid-like coccidians infecting the gallbladder epithelium of reptiles.

***Choleoeimeria ouachitaensis* McAllister, Seville, Connior, Trauth, and Robison, 2014**

Host: Ground skink, *Scincella lateralis*.
Locality: Ouachita Co.
Prevalence: 2/6 (33%).

***Isospora koberi* McAllister, Seville, Connior, Trauth, and Robison, 2014**

Host: *S. lateralis*.
Localities: Calhoun, Marion (type), Ouachita, and Union cos.
Prevalence: 2/5 (20%), 2/2 (100%), 1/6 (17%), and 6/29 (21%), respectively.

***Isospora scinci* Upton, McAllister, and Trauth, 1991**

Host: *P. fasciatus*.
Localities: Bradley, Marion, Van Buren (type) and Woodruff cos.
Additional host and locality: Broadhead skink, *Plestiodon laticeps*, Independence Co.
Prevalence: 1/1 (100%), 1/2 (50%), 1/1 (100%), and 2/5 (40%), respectively; *P. laticeps*: (25%).
Remarks: McAllister *et al*. (1994) added *P. laticeps* to the host list and 20 yrs later, McAllister *et al*. (2014) provided an additional report of *I. scinci* from *P. fasciatus* and extended its range into Oklahoma.

TEIIDAE

***Choleoeimeria (E.) sexlineatus* McAllister, Upton, and Trauth, 1991**

Host: Prairie racerunner, *Aspidoscelis sexlineatus viridis*.
Locality: Johnson Co.
Prevalence: 1/28 (4%).
Remarks: This is the only coccidian known from North American teiid lizards. It was originally placed in the genus *Eimeria* but developmental stages were clearly shown in gall bladder epithelium (Fig. 3 of McAllister *et al.* 1991) which places the coccidian in the genus *Choleoeimeria* sensu Paperna and Landsberg (1989).

OPHIDIA: COLUBRIDAE

***Eimeria arnyi* Upton and Oppert, 1991**

Host: Prairie ringneck snake, *Diadophis punctatus arnyi*.
Locality: Crawford and Marion cos.
Prevalence: 4/21 (19%).

***Eimeria attenuata* Wacha and Christiansen, 1974**

Hosts: Redbelly watersnake, *Nerodia erythrogaster*, broad-banded watersnake, *Nerodia fasciata confluens* western ribbon snake, *Thamnophis proximus proximus*.
Localities: Drew, Johnson, and Ouachita cos.
Prevalence: 2/20 (10%), 1/13 (8%), and 1/7 (14%), respectively.

***Eimeria conanti* McAllister and Upton, 1989**

Host: Mississippi green watersnake, *Nerodia cyclopion*, *N. erythrogaster*.
Locality: Mississippi Co.
Prevalence: 2/20 (10%).

***Eimeria cyclopion* McAllister, Upton, and Trauth, 1990**

Hosts: *N. cyclopion* (type), *N. erythrogaster*, *N. f. confluens*, diamondback watersnake, *Nerodia rhombifer*.
Locality: Mississippi Co.
Prevalence: 10/15 (67%), 1/1 (100%), 2/9 (22%), and 2/3 (67%), respectively.
Remarks: Oocysts of *E. cyclopion* degenerate rapidly, so it is recommended to measure and photograph specimens immediately after sporulation.

***Eimeria desotoensis* Upton, McAllister, and Trauth, 1992**

Host: Smooth earth snake, *Virginia valeriae elegans*.
Locality: Arkansas (see Duszynski and Upton 2009).
Prevalence: 1/3 (33%).

***Eimeria helmisophis* Wacha and Christiansen, 1974**

Hosts: Midwest worm snake, *C. a. helenae*, western worm snake, *Carphophis vermis*.
Localities: Carroll, Crawford, and Green cos.
Prevalence: 1/3 (33%) and 7/14 (50%).
Remarks: An *E. helmisophis*-like coccidian was reported from 3 taxa of watersnakes as follows: 5 of 20 (25%) *N. erythrogaster*, 3 of 13 (25%) *N. f. confluens*, and 11 of 30 (37%) *N. s. pleuralis* in Arkansas by

McAllister *et al*. (1995a). This coccidian is morphologically similar to oocysts of *E. helmisophis*. Duszynski and Upton (2009) listed this eimerian as a *species inquirenda*. However, studies utilizing DNA sequences will be necessary to elucidate significant differences between oocysts from worm snakes and watersnakes.

***Eimeria hobartsmithi* Upton, McAllister, and Trauth, 1992**
Host: *V. v. elegans*.
Locality: Arkansas (see McAllister *et al*. 1995a).
Prevalence: 1/3 (33%).

***Eimeria hydrophis* Wacha and Christiansen, 1974**
Host: *N. erythrogaster*, *N. s. pleuralis*.
Localities: Crawford, Franklin, Johnson, Little River, Madison, Poinsett, and Saline cos.
Prevalence: 11/20 (10%) and 9/30 (37%).

***Eimeria iowaensis* Wacha and Christiansen, 1974**
Host: *N. f. confluens*.
Locality: Poinsett Co.
Prevalence: 1/13 (8%).

***Eimeria kennicotti* Upton, McAllister, Trauth, and Gage, 1995**
Host: *D. p. arnyi*.
Locality: Polk Co.
Prevalence: 2/2 (100%).

***Eimeria natricis* Wacha and Christiansen, 1975**
Host: *N. erythrogaster*, *N. f. confluens*, midland water snake, *Nerodia sipedon pleuralis*, *T. p. proximus*.
Localities: Craighead, Crawford, Jackson, Little River, and Ouachita cos.
Prevalence: 2/20 (10%), 1/13 (8%), 2/30 (7%), and 1/7 (14%), respectively.

***Eimeria rhombifera* Upton and McAllister, 1988**
Host: *N. rhombifer*.
Locality: Mississippi Co.
Prevalence: 1/6 (17%).

***Eimeria septemvittata* Upton, McAllister, and Trauth, 1991**
Host: Queen snake, *Regina septemvittata*.
Locality: Johnson Co.
Prevalence: 3/3 (100%).

***Eimeria serpenticola* Upton and McAllister, 1990**
Hosts: *N. f. confluens*, *N. s. pleuralis*, *T. p. proximus*.
Localities: Drew, Marion, Poinsett, and Sharp cos.
Prevalence: 1/13 (8%), 3/30 (10%), and 1/7 (14%), respectively.

***Eimeria sipedon* Wacha and Christiansen, 1975**
Hosts: *N. erythrogaster*, *N. f. confluens*, *N. s. pleuralis*.
Localities: Crawford, Drew, Izard, Jackson, Johnson, Lonoke, Poinsett, Ouachita, Saline, and Sharp cos.
Prevalence: 11/20 (55%), 2/13 (15%), and 6/30 (20%), respectively.

***Eimeria striatula* Upton and McAllister, 1990**
Host: Rough earth snake, *Virginia striatula*.
Localities: Arkansas and Texas (see Duszynski and Upton 1989).
Prevalence: 12/32 (38%) combined.

***Eimeria tenuis* Upton and McAllister, 1988**
Hosts: *N. erythrogaster*, *N. rhombifer*, *N. s. pleuralis*.
Localities: Crawford, Mississippi, and Sharp cos
Prevalence: 1/20 (5%), 3/6 (50%), and 2/30 (7%), respectively.

***Eimeria zamenis* Phisalix, 1921**
Hosts: Southern black racer, *Coluber constrictor priapus*, western rat snake, *Pantherophis obsoletus*.
Localities: Arkansas (see Duszynski and Upton 2009).
Prevalence: 1/10 (10%) and 2/13 (15%).
Remarks: McAllister *et al*. (1995) reported this species as an *E. zamenis*-like coccidian. They further mentioned that it was doubtful this coccidian is the same species found in European colubrids, and we concur. Duszynski and Upton (2009) noted that this "species" could be best called a *species inquirenda*. Additional research is ongoing to help possibly unravel this enigma, including examination of endogenous stages and DNA sequencing.

***Isospora wilsoni* Upton, McAllister, Trauth, and Bibb, 1992.**
Host: *T. gracilis*.
Locality: Crawford Co.
Prevalence: 2/12 (17%).

GENUS *CARYOSPORA* (LÉGER, 1904) LÉGER, 1911
***Caryospora duszynskii* Upton, Current, and Barnard, 1984**
Hosts: Western coachwhip, *Coluber flagellum*, prairie kingsnake, *Lampropeltis calligaster calligaster*, speckled kingsnake, *Lampropeltis holbrooki*.
Localities: Conway, Franklin cos.

Prevalence: 1/3 (33%), 2/2 (100%), and 1/2 (50%), respectively.
Remarks: Modrý *et al.* (2005) demonstrated that mice (*Mus musculus*) are capable of indirectly transmitting infections of *C. duszynskii* to uninfected colubrid (rodent-feeding) snakes.

***Caryospora gracilis* Upton, McAllister, Trauth, and Bibb, 1992.**
Host: Flathead snake, *Tantilla gracilis*.
Locality: Crawford Co.
Prevalence: 1/12 (8%).

***Caryospora lampropeltis* Anderson, Duszynski, and Marquardt, 1968 (Fig. 5)**
Hosts: Eastern hognose, *Heterodon platirhinos*, *L. c. calligaster*, red milk snake, *Lampropeltis triangulum syspila.*
Localities: Ouachita; Lee and Saline cos; Lee Co.
Prevalence: 1/1 (100%), 1/6 (17%), 1/1 (100%), and 1/6 (17%), respectively.
Morphology/measurements: Oocysts (HWML 139321) were 25.6 × 21.3, a L/W ratio = 1.2, without a micropyle and oocyst residuum. Sporocysts measured 15.2 × 12.8, L/W ratio = 1.3, with Stieda and substieda bodies, and a sporocyst residuum. The outer wall of the oocyst was sculptured. These measurements and morphologies are similar to those in the original description of *C. lampropeltis* from *L. calligaster* from Illinois (Anderson *et al.* 1968) and those reported by McAllister *et al.* (2015) from *H. platirhinos* from Ouachita Co.

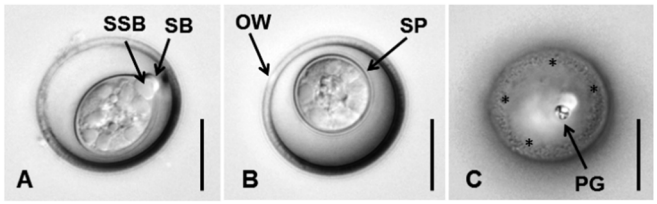

Figure 5. Oocysts of *Caryospora lampropeltis* from *Lampropeltis calligaster calligaster* from Saline Co. A. Oocyst showing Stieda body (SB) and substieda body (SSB). B. End-view of sporocyst (SP) showing oocyst wall (OW). C. Outer wall of oocyst showing sculptured appearance (*). Scale bars = 10 µm.

***Caryospora masticophis* Upton, McAllister, and Trauth, 1994**
Hosts: *C. flagellum* (type), *C. c. priapus.*
Locality: Saline Co.
Prevalence: 1/3 (33%) and 1/10 (10%).

VIPERIDAE

***Caryospora bigenetica* Wacha and Christiansen, 1982**
Hosts: Southern copperhead, *Agkistrodon contortrix contortrix*, timber rattlesnake, *Crotalus horridus*.
Localities: Polk Co.
Prevalence: 3/6 (50%) and 1/9 (11%).
Remarks: This species is pathogenic in secondary mammalian hosts (rodents, goats, dogs, pigs) and cause signs of clinical dermal coccidiosis, including markedly swollen facial tissue, ears, genitalia, and footpads (see Duszynski and Upton 2009).

CROCODYLIA: ALLIGATORIDAE

Coccidians have been reported from the American alligator, *Alligator mississippiensis* in southern Texas (McAllister and Upton 1990). However, none are known yet from this host in Arkansas.

Discussion

Interestingly, the latest phylogenetic studies on some coccidian parasites of lizards (e.g., caryosporans and isosporans) found evidence that support the polyphyletic origin of *Caryospora* and *Isospora* Megía-Palma *et al*. 2015). Their results suggest that these 2 genera are artificial generic names because taxonomic names are based on a group monophyletic origin. Until this finding has been completely resolved and accepted using life-cycle information, we are herein using the current generic nomenclature.

We have provided a summary of the Eimeriidae of the state herpetofauna. However, there are other coccidians that have been reported from Arkansas reptiles, including *Sarcocystis* spp. (Lindsay *et al*. 1991, 1992; Upton *et al*. 1995) but we are yet to find *Cryptosporidium* spp. in any amphibian or reptile from the state (see Upton *et al*. 1989; McAllister *et al*. 1995b). With over 58 species and subspecies of amphibians and 78 taxa of reptiles (Trauth *et al*. 2004), Arkansas supports a vast array of herpetofauna in diverse physiographic regions, many of which still need to be examined. Thorough and systematic surveys will surely increase the number of coccidians reported from the herptiles of Arkansas, including the possibility of discovering new host and geographic records as well as new species.

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Longitudinal patterns in an Arkansas River Valley stream: an Application of the River Continuum Concept

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Running Title: Longitudinal Patterns in an Arkansas River Valley Stream

Abstract

The River Continuum Concept (RCC) provides the framework for studying how lotic ecosystems vary from headwater streams to large rivers. The RCC was developed in streams in eastern deciduous forests of North America, but watershed characteristics and land uses differ across ecoregions, presenting unique opportunities to study how predictions of the RCC may differ across regions. Additionally, RCC predictions may vary due to the influence of fishes, but few studies have used fish taxa as a metric for evaluating predictions of the RCC. Our goal was to determine if RCC predictions for stream orders 1 through 5 were supported by primary producer, macroinvertebrate, and fish communities in Cadron Creek of the Arkansas River Valley. We sampled chlorophyll *a*, macroinvertebrates, and fishes at five stream reaches across a gradient of watershed size. Contrary to RCC predictions, chlorophyll *a* did not increase in concentration with catchment size. As the RCC predicts, fish and macroinvertebrate diversity increased with catchment size. Shredding and collecting macroinvertebrate taxa supported RCC predictions, respectively decreasing and increasing in composition as catchment area increased. Herbivorous and predaceous fish did not follow RCC predictions; however, surface-water column feeding fish were abundant at all sites as predicted. We hypothesize some predictions of the RCC were not supported in headwater reaches of this system due to regional differences in watershed characteristics and altered resource availability due to land use surrounding sampling sites.

Introduction

Aquatic systems are comprised of dynamic communities whose composition varies spatially, temporally, and in response to anthropogenic disturbance (Poff *et al*. 2006; Dodds *et al*. 2015). These communities are important for driving ecosystem processes critical for maintaining environmental health; that is, for energy (e.g. nutrients, carbon) to cycle through the ecosystem, biotic communities must interact with the changing environment to make sequestered resources available for use locally and downstream (Wallace and Webster 1996; Poff *et al.* 2006). This critical conjunction between biotic communities and the environment leads to broad, predictable relationships within a community (Dodds *et al.* 2015). The River Continuum Concept (RCC; Vannote *et al.* 1980) is the seminal framework that outlines how aquatic community structure is predicted to change as stream order increases. Fundamentally, it predicts shifts in community structure in response to the form of available energy. For example, in headwater streams, energy (in the form of carbon) is derived from allochthonous sources, such as leaf litter and fine particulates, that enter the stream. Here, communities are predicted to be dominated by organisms that are adapted to feeding on this external energy input and making it available to higher trophic levels.

These relationships, though first described in streams in eastern deciduous forests of North America, have been well studied and generally hold true in other ecoregions (Minshall *et al.* 1983). Some patterns, including macroinvertebrate community structure changes, can vary spatially due to landscape characteristics or riparian conditions (e.g. local land use). Stressors from urban and agricultural land use (e.g. increased conductivity and nutrient enrichment) may influence ecosystem function and structural changes along stream continuums (Paul and Meyer 2001; Walsh *et al*. 2005). Additional activities, such as unconventional natural gas (UNG) development, can contribute unique stressors to further alter ecosystem function and community structure (Johnson *et al*. 2015).

In this study, we examined how aquatic communities vary longitudinally in the Arkansas River Valley. Additionally, we wanted to expand upon the fish

predictions outlined in Vannote *et al*. (1980) by assigning functional feeding groups to fishes. To explore any deviations from the RCC patterns, we quantified stream quality using tolerance values, the Hilsenhoff biotic index, and percent Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa.

We predict the Cadron system will generally follow the patterns of the RCC. In regards to energy input, we predict chlorophyll *a*, a proxy for aquatic primary production and autochthonous energy input, will be inversely related to canopy cover and should generally increase with catchment area. Additionally, as catchment area increases we predict the ratio of coarse particulate organic matter (CPOM) to fine particulate organic matter (FPOM) will decrease.

We predict both macroinvertebrate and fish diversity will increase with increasing catchment area, but as a response to changes in available energy, we predict macroinvertebrates and fishes to respond differently according to their functional feeding group (FFG). We predict macroinvertebrate scrapers will vary with chlorophyll *a* and macroinvertebrates that feed on CPOM (shredders) will decrease with an increase in catchment area while those that feed on FPOM (collector-gatherers and collector-filterers) will increase. Macroinvertebrate predators will remain constant. Herbivorous fishes are predicted to vary with chlorophyll *a*. We predict that as catchment area increases macro-carnivore-piscivore fishes will increase. Benthic insectivore fishes (Ross 2013) are expected to increase in abundance while surface-water column insectivore fishes (Goldstein and Meador 2004) remain abundant but constant. Additionally, we predict fishes that do not disturb substrate will increase with increasing catchment area.

We predict any deviations from the patterns of the RCC could be explained by stream degradation and will be characterized by an abundance of highly tolerant macroinvertebrates, high Hilsenhoff values, and low EPT taxa.

Methods

Study Site

Cadron Creek (total drainage area = 437.7 km^2) confluences with the Arkansas River as a sixth order stream in Faulkner County, Arkansas. Upper portions of the watershed are characterized by riffle-pool structure as the stream flows south of the Boston Mountains and transitions to a lowland, meandering stream as it enters the Arkansas River Valley. We selected five sites in the upper Cadron Creek watershed to represent a range of stream orders (1-5) and catchment areas (4.1-360.0 km^2)(Figure 1 and Table 1). Sites were chosen based on stream accessibility and water availability. Consequently, not all sites lie on a contiguous body of water (Figure 1), but Minshall *et al.* (1983), the first comprehensive test of the RCC, had a similar discontinuous sampling method and was still able to detect predictable RCC patterns. All samples were taken between 23 September 2016 and 25 September 2016. Reach length varied from 161 to 336 m and contained 2 to 3 riffles and pools each.

For each site, we conducted a qualitative assessment, focusing on bank stability, riparian vegetative zone width, large wood abundance, and notable riffle characteristics. Overall, the majority of sites had fairly stable banks. Riparian vegetative zone width ranged from approximately 5 m at Site 1 to greater than 50 m at Site 2. Site 3 and Site 4 contained moderate amounts of large woody debris while Site 1, Site 2, and Site 5 had little. *Justicia americana* was present in riffles at all sites except Site 1.

We measured dissolved oxygen (DO; mg/L), specific conductivity (µS/cm), and temperature (°C) at each site using a YSI 85 handheld water quality meter (Yellow Springs Instruments Inc., Yellow Springs, OH). Stream width (0.1 m), depth (0.01 m), and dominant substrate (modified Wentworth Scale [Cummins 1962]: bedrock, boulder, cobble, pebble, gravel, sand/silt) were measured at five evenly spaced points along the three riffle transects at each site (n=15). Velocity (0.01 m/s) was measured using a Marsh-McBirney flow meter (FloMate 2000, Marsh-McBirney Inc., Frederick, MD) at five points in a single riffle transect per site. Discharge was calculated from the depth and velocity measurements.

Finally, we calculated percent land cover of forest, pasture, and developed land for each catchment using the National Land Cover Database 2001 (Homer *et al.* 2007) in ArcGIS 10.2.2 (ESRI, Redlands, California). All five sites were mostly forested catchments, ranging from 52% to 80%. Pasture was the second highest land use, ranging from 24% to 39% (Table 1).

Chlorophyll a *sampling*

We estimated canopy cover at each sampling point using a spherical crown densiometer (Table 1). We collected six periphyton samples at each site using a divot sampler (4.91 cm^2) following Lamberti and Steinman (1997). Across all riffles, two collections each were made at 25%, 50%, and 75% of wetted width. Water samples were filtered in the field with pre-weighed filters and kept on ice until laboratory analysis.

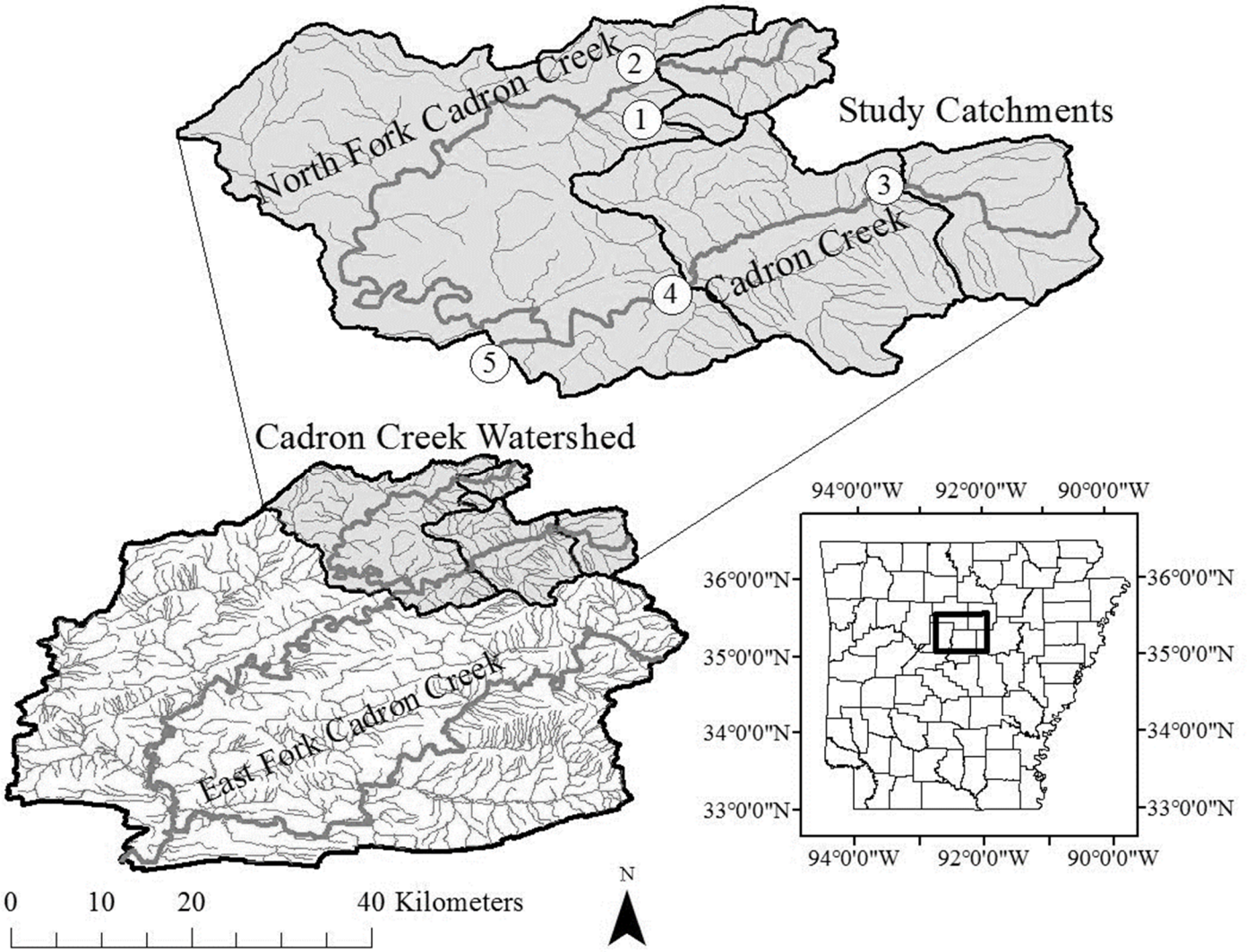

Figure 1. Locations of stream reaches sampled in the Cadrom Creek watershed. Upper portion of map corresponds to study catchments within the watershed and are labeled by increasing catchment area (see Table 1 for stream orders). Inset map to the right shows watershed within Arkansas.

Samples were typically collected from boulder or cobble substrate in riffles at depths ranging from 0.02 to 0.19 m.

We quantified chlorophyll *a* following Lamberti and Steinman (1997). Briefly, samples were extracted by soaking filters in acetone overnight at 4ºC in the dark. Following extraction, absorbance readings were taken at 664 and 750 nm using a Hach DR 5000 Spectrophotometer (Loveland, CO). We added 0.1 mL of 0.1M HCl and requantified absorbance at each wavelength. We calculated chlorophyll *a* concentration. using the equation provided by Lamberti and Steinman (1997), In total six samples from the five sites were excluded from analyses due to procedural errors.

Macroinvertebrate sampling

Six macroinvertebrate samples were haphazardly collected across two to three riffles at each site. To sample macroinvertebrates, we positioned a 25.4 x 30.5cm (L x W) 500µm mesh D-Frame dip net perpendicular to stream flow and disturbed sediments and macrophytes in a 0.5 x 0.5m area upstream of the net for one minute. Samples were combined to form a composite site sample, preserved in 70% ethanol, and transported back to the University of Central Arkansas for identification. Macroinvertebrates were identified using Merritt and Cummins (1996), McCafferty (1998), and Smith (2001). We identified individuals to the family level with the exception of families that contained multiple feeding groups and needed further distinction: in the family Tipulidae, we distinguished

Table 1. Stream habitat characteristics of sample sites in the Cadron Creek watershed. One standard deviation in parentheses. Land use data gathered from National Land Cover Database (2001).

	Site 1	Site 2	Site 3	Site 4	Site 5
Stream Order	1	3	3	4	5
Catchment Size (km^2)	4.1	17.2	38.1	127.4	360.0
GPS Coordinates	35° 28' 21.04" N 92° 13' 10.34" W	35° 29' 44.34" N 92° 13' 14.59" W	35° 26' 51.22" N 92° 7' 23.66" W	35° 24' 19.48" N 92° 12' 39.31" W	35° 22' 45.01" N 92° 17' 5.06" W
Land Use (%)					
Forest	66.0	80.0	64.0	52.0	55.0
Pasture	24.0	11.0	28.0	39.0	35.0
Developed	6.0	4.0	4.0	5.0	5.0
Riffle Depth (m)	0.03 (0.02)	0.06 (0.04)	0.06 (0.02)	0.07 (0.02)	0.13 (0.07)
Riffle Width (m)	2.3 (0.4)	6.3 (3.3)	6.2 (0.4)	5.8 (0.7)	17.7 (5.9)
Discharge (m^3/s)	0.006 (0.003)	0.030 (0.025)	0.013 (0.015)	0.15 (0.087)	0.80 (1.55)
Dominant Substrates	Boulder 40% Cobble 33%	Pebble 47% Gravel 33%	Cobble 47% Sand 20%	Pebble 53% Cobble 27%	Cobble 47% Boulder 20%
Temperature (°C)	25.10	21.20	24.60	23.0	23.60
Conductivity (μS/cm)	50.70	21.30	34.50	37.20	48.40
Dissolved Oxygen (mg/L)	3.70	6.71	6.30	6.09	6.29
Chlorophyll α ($\mu g/cm^2$)	1.7 (1.63)	6.07 (3.71)	4.99 (4.01)	7.84 (5.55)	6.39 (8.77)
Canopy Cover (%)	81.5 (11.04)	88.08 (6.99)	52.2 (17.86)	88.67 (3.68)	74.54 (12.90)

Hexatoma; in the family Chironomidae, we distinguished the sub-family Tanypodinae. Isopoda and Amphipoda were only identified to order. We assigned functional feeding groups (FFG) following Merritt and Cummins (1996), though Pennak (1978) was used for Isopoda and Zilli *et al.* (2008) for *Corbicula*. We used FFG assignments and the ratio of shredders to total collectors as a proxy to estimate the CPOM/FPOM ratio following Merritt *et al.* (2002).

Fish sampling

We opportunistically sampled fishes in all available habitat generally following Matthews (1986, 1990) using one 1.2 x 4.6m seine in pools/runs and one 1.2 x 2.4m seine in riffles (5 mm mesh). Two crews simultaneously sampled riffles and pools/runs for 50 to 80 minutes, with time varying due to reach length. Fishes were fixed in 10% formalin then identified in the laboratory at the University of Central Arkansas.

Functional feeding groups were assigned to fishes following Matthews (1998) and Ross (2013). Species assigned to multiple feeding groups were split into equivalent proportions following Greathouse and Pringle (2006). Furthermore, fishes were divided into two feeding modes based on substrate disturbance: those that mechanically disturb substrate and those that do not disturb the substrate (Matthews 1998).

Environmental quality assessment

To estimate stream quality across our sites, we used taxonomic tolerance values from appendix B of the EPA Rapid Bioassessment Protocol (Barbour *et al.* 1999) for the macroinvertebrates we collected. Using these values, we estimated organic pollution for each stream using the biotic index equation proposed by Hilsenhoff (1982). In theory, site degradation should be inversely related to the number of sensitive taxa, so as this number increases (and the ratio of tolerant taxa increases), so does pollutant abundance. To complement this metric, we calculated percent Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa, which are the most sensitive orders, as a proportion of total individuals collected.

Statistical analyses

Statistical significance was determined at $p < 0.05$ and all analyses were conducted in R (version 3.3.2; R Core Team 2016). We used Pearson's correlations (r) to examine relationships between habitat and biotic variables. If assumptions of normality were violated, we $\log_{10}$-transformed the data. In cases where transformation did not correct normality, we used Spearman's rank correlation (r_s). Correlations were performed using the *rcorr* function in the Hmisc package (Harrell Jr 2015). We used ANOVA to determine if chlorophyll *a* differed between sites. To test for differences in FFG abundances between sites, we used the G-test of independence in the DescTools package (Signorell 2016); pairwise G-tests with alpha levels adjusted for multiple comparisons in the RVAideMemoire package (Hervé 2016) were used for post hoc analyses. To test how taxon sensitivity varied both within and between sites, we created three bins: the first bin we called "sensitive" and included taxa with tolerance values less than or equal to 4; the second bin was "moderately tolerant" and included taxa with tolerance values above four but less than or equal to 6; and the third bin was "tolerant" and included all taxa with tolerance values above 6. We used the G-test of independence with pairwise G-tests to test for differences between bins.

Results

Habitat

Site 1, Site 2, Site 3, and Site 4 were relatively narrow (< 6.3 m) with low discharge. Width was approximately three times larger at Site 5 (Table 1). Dissolved oxygen was moderately high at most sites (73% - 78% saturation) but was relatively low (46% saturation) at Site 1. Riffles were mostly dominated by cobble and pebble, but gravel and sand were major components at Site 2 and Site 3. Canopy cover was relatively high at most sites (74.5% to 88.7%) but lower at Site 3 (52.2%).

Catchment area was positively correlated with discharge ($r = 0.93$, $p = 0.02$), and riffle depth ($r = 0.91$, $p = 0.03$). Catchment area was not significantly correlated with other habitat variables.

***Chlorophyll* a**

Chlorophyll *a* was on average lowest at Site 1 and higher at all other sites, though there was no statistical difference between sites ($F_{4,19} = 1.39$, $p = 0.28$). Chlorophyll *a* concentration was not significantly correlated to canopy cover ($r = 0.20$, $p = 0.75$). However, chlorophyll *a* was significantly correlated with average sample depth ($r = 0.88$, $p = 0.048$) and trended to increase with catchment area ($r = 0.80$, $p = 0.1$) and discharge ($r = 0.75$, $p = 0.15$).

Macroinvertebrate

We collected 4,266 individuals across 38 taxa (Table 2). As expected, Shannon's diversity ranged from 0.95 to 2.31 and increased with catchment area ($r = 0.98$, $p < 0.01$); taxon richness ranged from 10 to 26 and increased with catchment area ($r = 0.95$, $p = 0.02$).

We expected scraper abundance to vary with periphyton concentration. Scraper relative abundance differed between sites ($G_4 = 18.47$, $p < 0.01$; Figure 2), but neither absolute ($r = 0.6$, $p = 0.28$) nor relative ($r = 0.6$, $p = 0.29$) scraper abundance was correlated with canopy cover nor periphyton concentration (absolute scraper: $r = 0.33$, $p = 0.59$; relative scraper: $r = -0.06$, $p = 0.92$).

Not all FFGs varied with catchment area as predicted by Vannote *et al*. (1980). Shredder relative abundance differed between sites ($G_4 = 209.84$, $p < 0.01$), where relative abundance was highest at Site 1, and did not differ among other sites (Figure 2). Collector (filterers and gatherers collectively) relative abundance differed between sites ($G_4 = 81.03$, $p < 0.01$), where Site 1 was significantly lower than all other sites, none of which differed from each other. Specifically, collector-

Table 2. Macroinvertebrate taxa total abundance at five sites in the Cadron Creek watershed. NT= Non-Tanypodinae NH= Non-*Hexatoma*.

Taxon	Site 1	Site 2	Site 3	Site 4	Site 5
			Filtering Collectors		
Corbicula	——	——	——	82	11
Hydropsychidae	9	61	79	117	190
Isonychidae	——	——	1	42	38
Philopotamidae	5	51	140	89	72
Simuliidae	——	14	——	10	28
Sphaeriidae	——	——	37	——	1
			Gathering Collectors		
Amphipoda	——	——	——	1	——
Baetidae	——	5	4	50	44
Caenidae	1	——	3	1	1
Chironomidae (NT)	54	466	456	146	148
Elmidae	1	62	88	344	80
Ephemeridae	——	——	——	14	——
Hydrophilidae	——	——	——	——	2
Leptoceridae	——	——	1	——	——
Leptophlebiidae	——	——	——	1	1
Oligochaeta	——	——	1	——	——
			Predators		
Aeshnidae	——	——	——	1	——
Calopterygidae	——	——	15	——	——
Chloroperlidae	——	——	——	1	——
Coenagrionidae	——	1	3	——	——
Corydalidae	——	3	2	2	5
Dytiscidae	——	——	——	——	1
Gomphidae	——	1	4	3	3
Gyrinidae	——	——	1	——	——
Hexatoma	3	10	3	8	15
Perlidae	——	——	——	1	22
Perlodidae	——	——	——	12	——
Rhyacophilidae	——	5	——	——	——
Sisyridae	——	——	——	1	——
Tabanidae	——	——	——	——	2
Tanypodinae	16	67	184	87	7
Veliidae	——	——	——	——	9
			Scrapers		
Heptageniidae	54	14	1	109	46
Psephenidae	——	——	——	3	4
			Shredders		
Haliplidae	——	——	1	2	——
Isopoda	436	1	1	4	2
Lepidoptera	——	2	2	——	——
Lepidostomatidae	——	——	——	——	1
Tipulidae (NH)	5	7	4	13	4

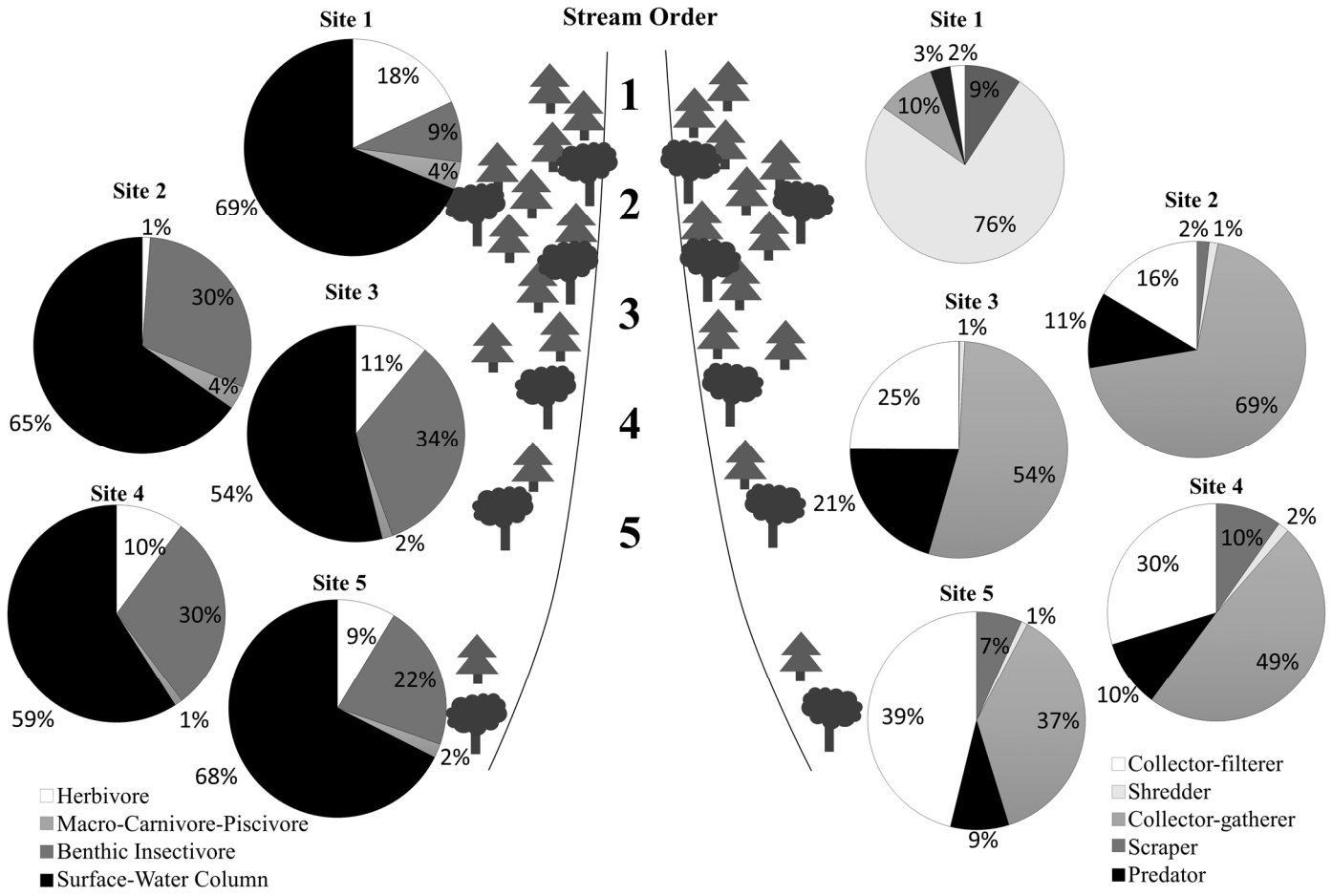

Figure 2. Relative abundance of fish (left) and macroinvertebrate (right) functional feeding groups at 5 sites along Cadron Creek.

gatherer relative abundance differed between sites (G_4 = 55.37, $p < 0.01$); this group was least abundant at Site 1 and decreased on average across other sites with increasing catchment area (Figure 2). Collector-filterer relative abundance differed between sites (G_4 = 52.24, $p < 0.01$), where they were least abundant at Site 1 and increased on average across all other sites with increasing catchment area (Figure 2). Relative predator abundance was more variable (range 3.3% - 20.6%) than expected and differed between sites (G_4 = 14.76, $p < 0.01$), contrary to our prediction. Site 1 had significantly fewer predators than Site 3, but no other site pairings differed from each other (Figure 2).

CPOM/FPOM ratio, estimated from the ratio of shredders to collectors, differed between sites (G_4 = 19.67, $p < 0.01$), where Site 1 had the highest CPOM/FPOM ratio while the other sites did not differ from each other.

Sensitivity bins differed within sites. At Site 1 (G_4 = 802.44, $p < 0.01$), Site 2 (G_4 = 578.73, $p < 0.01$), and Site 3 (G_4 = 791.89, $p < 0.01$), individuals from tolerant taxa were the most abundant, followed by individuals from sensitive taxa, with only a few individuals from moderately tolerant taxa being represented. Conversely, at Site 4 (G_4 = 956.62, $p < 0.01$) and Site 5 (G_4 = 458.68, $p < 0.01$), individuals from sensitive taxa were the most common, followed by individuals from tolerant taxa, and then individuals from moderately tolerant taxa. EPT ranged from 4.1% at Site 1 to 56.3% at Site 5. Similar to the pattern observed with the tolerance data, percent EPT was significantly different across sites (G_4 = 42.79, $p < 0.01$), where values were low at Site 1 and Site 2 and high at Site 4 and Site 5.

Hilsenhoff's Index values suggest all sites have mild organic pollution; however, water quality improved with catchment area. Site 5 and Site 4 were determined to be in "good" quality, but could still contain some organic pollution. Both Site 3 and Site 2 were "fair" in quality, indicating both streams contain a fairly significant amount of organic pollution. Site 1 was

in "fairly poor" condition and is predicted to have significant amounts of organic pollution.

Fish

We collected 811 individuals across 8 families and 25 species (Table 3), a comparable number of individuals to previous sampling events (SR Adams, unpublished data). As expected, diversity ranged from 2.02 to 2.47 and increased with catchment area ($r = 0.97$, $p < 0.01$); species richness ranged from 6 to 21 and trended to increase with catchment area ($r = 0.80$, $p = 0.09$). We found an addition of 7 taxa (*Cyprinella whipplei*, *Notropis boops*, *Notropis greenei*, *Pimephales notatus*, *Hypentelium nigricans*, *Gambusia affinis*, and *Etheostoma zonale*) at the two most downstream sites (Site 4 and Site 5). Three taxa (i.e., *Campostoma anomalum*, *Fundulus olivaceus*, and *Etheostoma spectabile*) were widely distributed and found at all sites.

Abundance of benthic insectivores differed among sites ($G_4 = 18.37$, $p < 0.01$), with Site 1 having the lowest relative abundance. No other functional feeding groups differed in relative abundance between sites.

Abundance of herbivores was not significantly correlated to chlorophyll *a* concentrations ($r = -0.66$, $p = 0.22$). Surface/water-column feeders were the most abundant FFG at all sites and ranged in relative abundance from 53.00 to 66.80 ($\bar{x} = 62.42$). *Fundulus olivaceus* relative abundance had a negative relationship with catchment area ($r = -0.96$, $p < 0.01$); whereas *Labidesthes sicculus* increased with catchment area, but was not significant ($r = 0.83$, $p = 0.08$). Omnivores were not abundant and were only collected at Site 3 and Site 4 (Table 3).

Fishes that do not physically disturb the substrate were most abundant for all sites and ranged in relative abundance from 54.50 to 70.80 ($\bar{x} = 64.02$). Substrate disturbers ranged in relative abundance from 29.00 to 45.40 ($\bar{x} = 35.90$). Substrate disturbers and non-disturbers relative abundance did not differ between sites ($p > 0.50$).

Discussion

The RCC (Vannote *et al.* 1980) outlines predictable changes in ecosystem community structure as the available forms of energy change. In an undisturbed landscape, energy enters headwater streams allochthonously, typically in the form of detritus. Organisms found in these headwater areas are adapted to using this energy, and through their processing, coarse detrital input changes energy forms and becomes available for other organisms. In the Cadron system of Arkansas, though, we observed an abundance of autochthonous energy input in the headwater streams.

Chlorophyll *a* concentrations were higher than we would expect based on the amount of available light, as measured by canopy cover. Interestingly, canopy cover was not a predictor of chlorophyll *a* concentrations in our system. Instead, all of our sites had higher average chlorophyll *a* values than streams with comparable catchment areas in the Arkansas River Valley (e.g. Austin *et al.* 2015), and could be categorized as moderately eutrophic (Barbour *et al*. 1999). Sampling bias could explain our high concentrations if we sought substrates that had visible periphyton, but this seems unlikely.

More plausible is the detected eutrophication is a direct result of the streams being in close proximity to pastures and the abundance of UNG wells in the area (2.14 well/m^2 in the watershed). Runoff from pastures (Smart *et al*. 1985; Lohman *et al*. 1991) and UNG wells (Austin *et al*. 2015; Johnson *et al.* 2015) have been shown to be related to increased chlorophyll *a* concentration. Further, macroinvertebrate community structure indicated fairly significant levels of pollution, connected to eutrophication in these low order streams, as indicated by the Hilsenhoff index.

For example, sensitive fish and macroinvertebrate taxa that were present in the two largest stream reaches were not detected in the three smallest streams we sampled. These lowest order streams also exhibited the lowest fish and macroinvertebrate diversity, as the RCC predicts (Vannote *et al*. 1980), but macroinvertebrate taxa that feed on detrital input (i.e. shredders) predicted to be present were detected only at the upstream-most site. *Campostoma anomalum*, a herbivorous fish, was unexpectedly abundant at Site 1, likely suggesting that the eutrophication at Site 1 supports periphyton growth, which *C. anomalum* feed on (Power and Matthews 1983; Power *et al*. 1988; Gelwick *et al.* 1997).

Abundance of sensitive taxa increased at the two largest stream reaches. Likely, the effects of pasture and UNG pollution runoff is either being buffered in these reaches by the larger riparian zones surrounding these streams or have a lesser influence on biotic communities due to dilution. These larger reaches had community structures more similar to those predicted by Vannote *et al.* (1980) as well. Diversity for macroinvertebrates and fishes were highest at these sites. Additionally, for macroinvertebrates, shredder, scraper, collector, and predator abundances followed RCC predictions. We did not find support for our hypothesis that fish predator abundance would increase with catchment area, but this

Table 3. Fish taxa total abundance at five sites in the Cadron Creek watershed. Fish that were classified into more than one functional feeding group were split evenly between groups (i.e. *Lepomis cyanellus, L. macrochirus, L. megalotis*).

Species	Site 1	Site 2	Site 3	Site 4	Site 5
			Herbivore*		
Campostoma anomalum	22	1	7	31	21
			Benthic Insectivore*		
Hypentelium nigricans	——	——	——	2	——
Noturus exilis	——	3	5	27	4
Lepomis cyanellus	5	1	——	0.67	——
Lepomis macrochirus	5	1.5	——	8	3
Lepomis megalotis	——	4.5	1.5	22	7
Lepomis microlophus	——	——	2	1	1
Etheostoma blennioides	——	——	3	1	17
Etheostoma flabellare	——	1	3	11	2
Etheostoma nigrum	——	1	2	3	——
Etheostoma spectabile	1	9	5	7	2
Etheostoma whipplei	——	3	——	6	2
Etheostoma zonale	——	——	——	2	14
Percina maculata	——	1.5	——	——	0.5
			Omnivore*		
Ameiurus natalis	——	——	1	——	——
			Surface-Water-Column†		
Cyprinella whipplei	——	——	——	1	8
Lythrurus umbratilis	6	4	9	17	——
Notropis boops	——	——	——	15	——
Notropis greenei	——	——	——	——	19
Labidesthes sicculus	——	17	8	44	96
Fundulus olivaceus	68	26	16	59	27
Gambusia affinis	——	——	——	7	——
Lepomis cyanellus	5	1	——	8	3
Lepomis macrochirus	5	1.5	——	8	3
Lepomis megalotis	——	4.5	1.5	22	7
Percina maculata	——	1.5	——	——	0.5
			Macro-Carnivore-Piscivore†		
Lepomis cyanellus	5	1	——	0.67	——
Micropterus punctulatus	——	1	——	3	5
Micropterus salmoides	——	1	1	——	——
			Omnivore†		
Pimephales notatus	——	——	——	1	——

*Physically disturbs substrate
†Does not physically disturb substrate

is generally an expected consequence when seining for large-bodied fishes (Jackson and Noble 1995).

Although few RCC predictions for fish were observed, we found an interesting pattern in distribution and abundance of two surface/water-column species that have different feeding habits. One surface/water-

column feeder (*Fundulus olivaceus*) decreased in relative abundance with increased catchment area while *Labidesthes sicculus* trended towards an increase. This finding is consistent with other studies (Guillory 1982; Porter and Patton 2015), but functional feeding groups and sources of food items were not discussed as drivers of upstream/downstream distribution and abundance patterns. *Fundulus olivaceus* derives the majority of its diet from terrestrial input (Ross 2001; Matthews *et al*. 2004) and is presumably less dependent on flow for drifting food items. *Labidesthes sicculus* is a water-column particulate feeder (Matthews 1998) and also has a diet highly comprised of chironomids (Ross 2001), a taxon predicted to increase with catchment area (Vannote *et al.* 1980). This suggests a shift from direct consumption of terrestrial invertebrates in headwaters to more utilization of aquatic invertebrates, ultimately assimilating organics transported from upstream and/or autochthonous primary production, in downstream reaches, supporting RCC predictions.

Overall, local anthropogenic alterations to the surrounding catchment appear to influence headwater community structure and function. Generally, most fundamental RCC predictions were supported, with a few minor deviations in our headwater reaches, where pollution indices were highest. The small headwater streams in this system have an overrepresentation of primary producers relative to the predictions in Vannote *et al.* (1980), potentially suggesting local nutrient enrichment (Lohman *et al*. 1991). Similarly, macroinvertebrate and fish communities support this idea; sensitive taxa that perform important ecosystem functions are noticeably absent from these reaches.

Although surrounding land use promoted more primary production than expected in the smaller catchments, the basic predictions of the RCC hold, such that there is a shift, albeit exaggerated, from predominantly shredding taxa in the headwaters to collecting taxa downstream. Considering the RCC predicts a dynamic equilibrium between available nutrients and community structure, more samples from different seasons and additional sites, especially lower order streams, in the Cadron system would help provide a complete picture of the RCC in this region.

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We would like to thank Jacob Waymack and Sam Massery for assisting with fish collection, the landowners that allowed us access to their properties, Arkansas Game and Fish Commission for collecting permits, and three anonymous reviewers for their insightful comments. We appreciate Dr. Sally Entrekin for her advice and use of equipment during this project.

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Lithologic Stratigraphic Position, Sequence and Diagenetic History, Lower Mississippian Tripolitic Chert, Northern Arkansas and Southern Missouri

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Running title: Lower Mississippian Tripolitic Chert Stratigraphy

Abstract

Tripolitic chert development in the southern Ozark region is associated with a third-order, transgressive-regressive cycle comprising St. Joe transgressive packstones, succeeded by lower Boone calcisiltites, with black, penecontemporaneous, nodular chert deposited during maximum flooding, overlain by basal upper Boone calcisiltites deposited during highstand. The onset of regression produced upper Boone packstones and grainstones with white-gray, later diagenetic chert reflecting groundwater replacement along bedding planes. Tripolitic chert is a product of the highstand calcisiltites at the base of the upper Boone Formation of Arkansas, and its equivalent, the Elsey Formation of southern Missouri. This tripolitic chert appears to reflect a hydrothermal event likely occurring after the emplacement processes of both Boone cherts that had ended by Chesterian time. After hydrothermal silicification, the interval experienced groundwater removal of most of the remaining carbonate leaving open porosity characteristic of tripolitic chert. A second hydrothermal event precipitated terminated and doubly terminated quartz crystals as well as quartz druse in the cavities produced by the earlier carbonate leaching from the tripolitic chert. Timing of the hydrothermal events is not clear, but they may reflect lateral secretion produced by the Ouachita Orogeny in the late Pennsylvanian.

Introduction

Tripolitic chert is a microcrystalline, porous form of sedimentary quartz (SiO_2), resulting from the alteration of chert or novaculite, or by the leaching of highly siliceous limestones (Tarr 1938). In northern Arkansas and southern Missouri, tripolitic chert can be found within the basal portion of the upper Boone Formation, which corresponds to the highstand interval of a third-order, transgressive-regressive sequence stratigraphic cycle. According to Tarr (1926), the initial presence of disseminated carbonate within the chert is essential for tripolitic chert formation. The silica replacing the calcisiltites of the basal upper Boone Formation was likely emplaced by hydrothermal fluids produced by the Ouachita Orogeny in the late Pennsylvanian. A second hydrothermal event precipitated terminated quartz crystals in some of the void spaces left by decalcitization.

Geologic Setting

In the southern midcontinent, most of the Paleozoic and Mesozoic section reflects eustatic cycles of transgression and regression by epeiric seas in a cratonic setting. This resulted in the deposition of thin lithostratigraphic units of a variety of sedimentary lithologies, including both marine and non-marine sediments. These sedimentary units dip radially away from the Ozark Dome, which is a broad cratonic uplift cored by Precambrian granite and rhyolite centered in southeastern Missouri (Chinn and Konig 1973). Limestones dominate the rock record until the Pennsylvanian, when clastic sequences of sandstone and shale suppressed carbonate deposition (Manger *et al.* 1988).

Lithostratigraphy and Sequence Stratigraphy

The limestones comprising the Lower Mississippian Boone Formation were produced on a broad, shallow carbonate platform called the Burlington Shelf (Lane 1978) and were subsequently transported down-ramp and deposited. This interval reflects a single, third order, transgressive-regressive cycle bounded by regional unconformities. The transgressive interval is reflected by the primarily chert-free limestones of the St. Joe Formation. The lower Boone, referred to as the Reeds Spring Formation in southern Missouri (Figure 1), reflects the maximum flooding interval (Manger and Shelby 2000). This unit comprises calcisiltites and contains penecontemporaneous chert, which is dark, nodular and disrupts the bedding of the limestone,

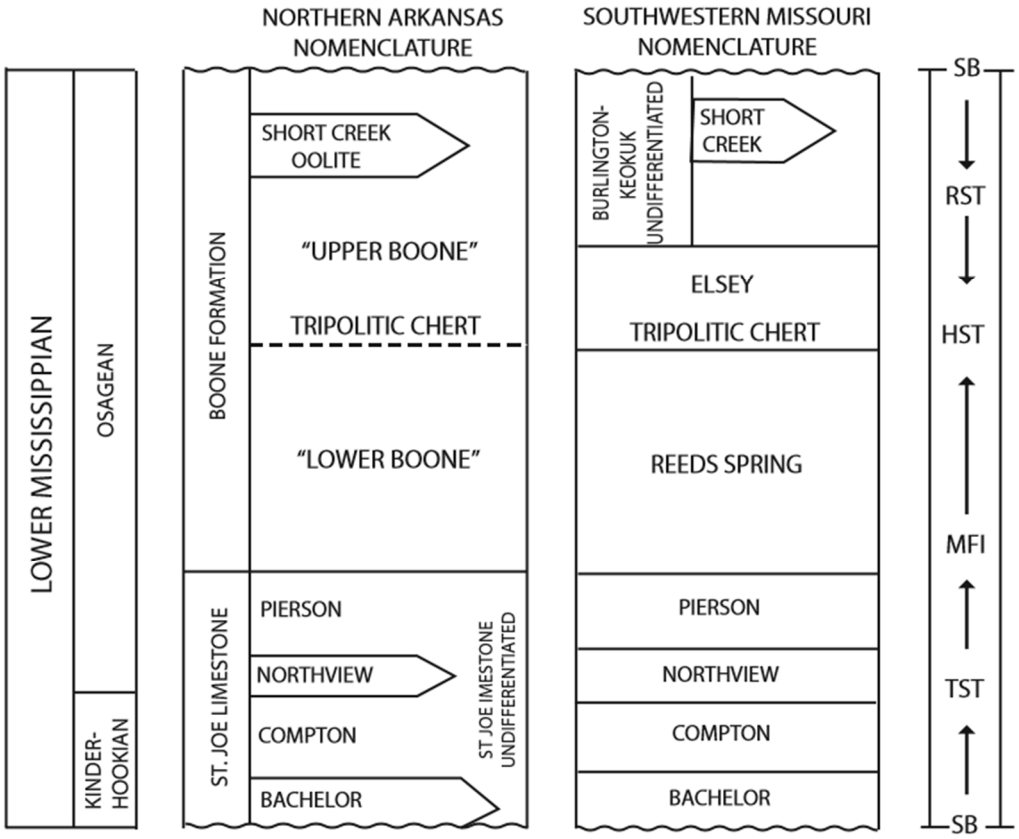

Figure 1. Stratigraphic column representing northern Arkansas and southern Missouri, modified from Manger and Thompson, 1982.

Figure 2. Roadcut along US Highway 71, near Bella Vista, displaying the upper Boone Limestone. Lowest beds are tripolitic chert.

exhibiting compaction features, indicating deposition prior to lithification of the surrounding limestone (Manger and Thompson 1982). The upper Boone marks the highstand and regressive interval and consists of sand to gravel size bioclastic grains (Shelby 1986), usually crinozoan detritus (McFarlin 2016). This interval contains later diagenetic chert, which is white and clearly a replacement of carbonate grains by silica following bedding planes, and favoring the finer grained intervals due to a greater surface area. The groundwater replacement of carbonate grains in the upper Boone must have preceded the unconformity between the Osagean upper Boone and Chesterian Hindsville Limestone, evident by a chert breccia in the Hindsville Limestone containing later diagenetic chert clasts.

The tripolitic chert in northern Arkansas and southern Missouri is confined stratigraphically to the lower portion of upper Boone and its equivalents (Elsey Formation in Missouri, see Figure 1). The chert in this interval is the result of hydrothermal replacement of carbonate by silica, producing massive, white, very fine-grained chert with disseminated carbonate between the lower Boone and the upper Boone (Figures 2, 3). This chert replacement left pseudo-nodular limestone bodies (Figure 4). It then becomes tripolitic as groundwater dissolves the remaining carbonate within the chert, creating porosity (Figures 5, 6).

Discussion

The tripolitic chert of northern Arkansas and southern southern Missouri is characterized by its porous texture

(Figures 5, 6) caused by the decalcitization of the remaining carbonate grains in the fine-grained chert of the basal upper Boone.

Figure 3. View looking south from the tripolitic chert exposure toward the Boone (= Reeds Spring) with penecontemporaneous chert.

Figure 4. Photograph of upper Boone outcrop displaying pseudo-nodular limestone bodies (gray) surrounded by tripolitic chert (white).

The occurrence of the tripolitic chert at the basal upper Boone is the consequence of an isolated hydrothermal event. Hydrothermal, silica-rich fluid, possibly reflecting lateral secretion produced by the Ouachita Orogeny in the late Pennsylvanian, was confined by the penecontemporaneous chert below and the upper Boone above (Figures 2, 3). These formations are nearly impermeable and acted as a confined aquifer, allowing the movement of hydrothermal fluids between the two layers, but preventing the migration either up or down in the formations. This hydrothermal replacement leaves pseudo-nodular limestone bodies surrounded by massive, white, very fine-grained chert (Figures 4, 6).

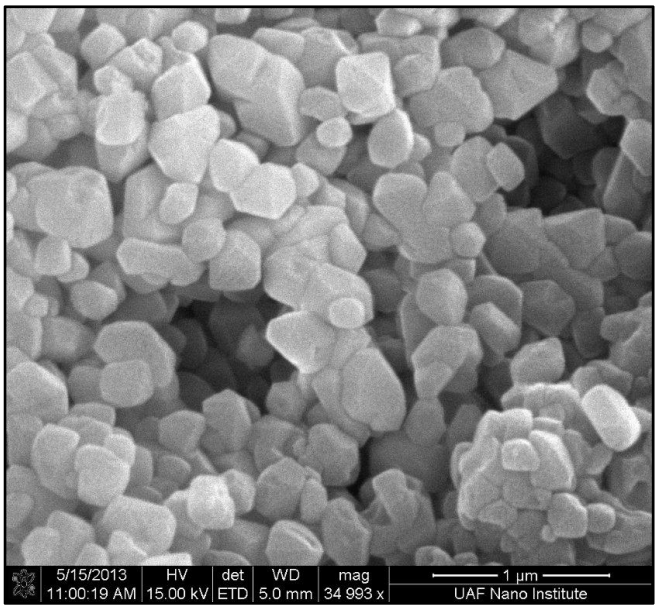

Figure 5. Scanning electron microscopy shows fine-grained, crystalline character of the silica and porosity within the chert.

Conclusions

The presence of terminated and doubly terminated quartz crystals within the cavities of the tripolitic chert strongly indicates two hydrothermal events in this interval. The first hydrothermal event occurred within the lower portion of the upper Boone formation, creating the very fine-grained chert. Groundwater decalcitized this fine-grained chert, leaving void spaces, which created the tripolitic chert. Terminated quartz crystals within those pore spaces indicates a second, silica-rich, hydrothermal event that possibly emplaced the lead-zinc deposits of the southern Ozarks.

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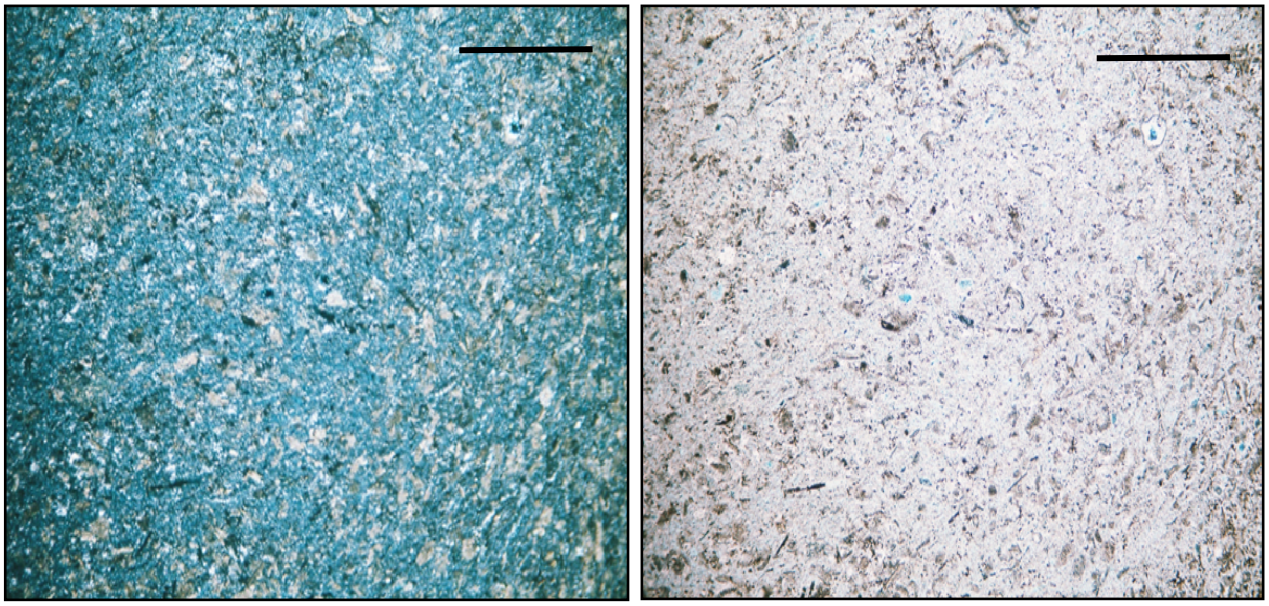

Figure 6. Thin section of tripolitic chert. Left image displays sample under crossed nicols, right image displays sample under plane light. Note abundant pin-point porosity (blue areas) from decalcitization in right image. Scale bar: 5mm.

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Hydrothermally Emplaced, Lower Mississippian, Tripolitic Chert and Its Possible Relationship to the Tri-State Lead-Zinc Mining District

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Running title: Tripolitic Chert and its Possible Relationship to the Tri-State Lead-Zinc Mining District

Abstract

Across the southern Ozark Region, northern Arkansas, southwestern Missouri, and northeastern Oklahoma, exposures of the Lower Mississippian Boone Formation and its equivalents exhibit well-developed tripolitic chert that has been mined, more or less continuously, for at least 80 years. The tripolitic chert is a replacement of an interval within the basal portion of the upper Boone Formation in Arkansas and Oklahoma, and equivalent to the Elsey Formation in Missouri. The movement of silica-rich, hydrothermal fluids appears to have been much like that of a confined aquifer. It followed the basal upper Boone Formation (Arkansas) = Elsey Formation (Missouri) and was bound below by an impermeable interval at the top of the lower Boone Formation (Arkansas) = Reeds Spring Formation (Missouri), and above by the base of the upper Boone Formation (Arkansas) = Burlington-Keokuk (Missouri). The first hydrothermal event incompletely silicified the basal upper Boone = Elsey Formation. After leaching of the remnant carbonate, thus forming the tripolitic chert, a second hydrothermal event deposited terminated and doubly terminated quartz crystals, and druse in the tripolitic chert voids. This hydrothermal event may have produced the Mississippi Valley-Type (MVT) lead-zinc deposits in northeast Oklahoma and southwestern Missouri. The famous deposits at Picher, Oklahoma, and Joplin, Missouri, appear to be positioned in the apparent path of the hydrothermal fluid migration. While timing of these hydrothermal events is unclear, they may reflect lateral secretion produced by the Ouachita Orogeny in the Late Pennsylvanian.

Pulses of Hydrothermally Emplaced Silica: Terminated and Doubly-Terminated Quartz Crystals Filling Tripolitic Chert Secondary Porosity

Tripolitic chert and euhedral quartz druse found within the tripolitic chert indicate at least two pulses of hydrothermal activity in the southern Ozark region. The initial hydrothermal fluids replaced the fine-grained calcisiltites of the basal upper Boone Formation (Arkansas) = Elsey Formation (Missouri) producing a very fine-grained, white chert interval with remnant, pseudo-nodular masses of unaltered calcisiltites (Figure 1).

Figure 1. Basal upper Boone outcrop of tripolitic chert with pseudo-nodular, but unaltered, calcisiltite bodies (gray) surrounded by tripolitic chert (white); Pineville, Missouri, roadcut; hammer for scale.

This interval between the top of the lower Boone Formation (Arkansas) = Reeds Spring Formation (Missouri) and the base of the upper Boone Formation (Arkansas) = Burlington-Keokuk (Missouri) was then exposed to groundwater invasion that dissolved most of the carbonate remaining in the very fine-grained, white chert. The resulting porous, siliceous lithology was designated tripolitic chert (Tarr 1938) (Figure 2).

Further examination of the porosity of the tripolitic

chert using scanning electron microscopy (SEM) has revealed terminated and some doubly terminated quartz crystals as well as druse filling some of these cavities (Figures 3, 4). Presence of the quartz druse suggests a second pulse of hydrothermal fluids passing through the basal upper Boone (Arkansas) = Elsey Formation (Missouri).

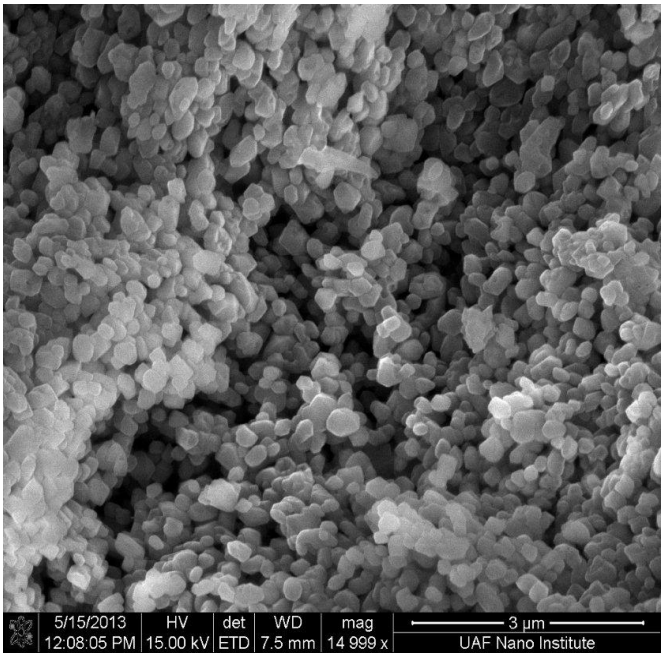

Figure 2. SEM image of tripolitic chert from the Pineville roadcut in Figure 1. Note porosity and very high magnification (image from Minor, 2013).

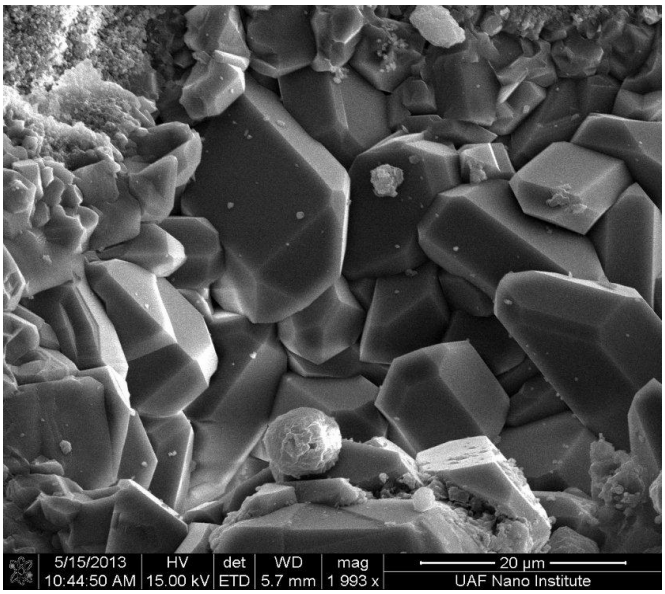

Figure 3. SEM image of terminated quartz crystals in a cavity within the tripolitic chert from the Pineville roadcut in Figure 1 (image from Minor, 2013).

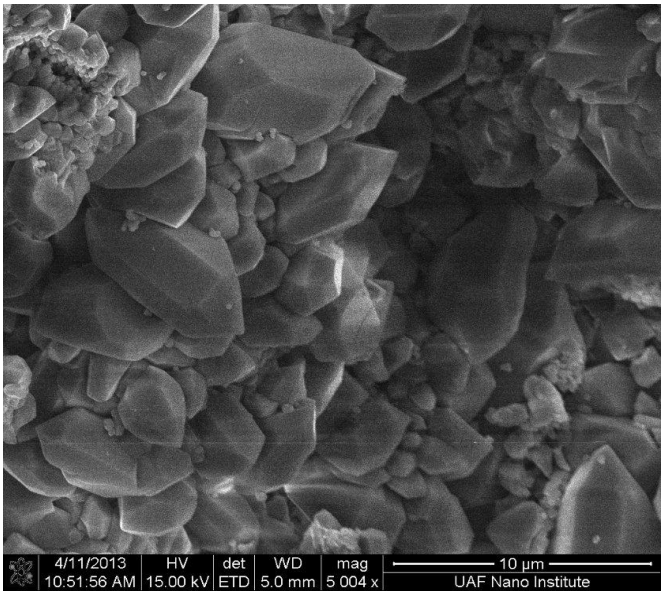

Figure 4. SEM image of quartz crystals, some with double terminations, in a cavity within the tripolitic chert from the Pineville roadcut in Figure 1 (image from Minor, 2013).

Source of the Silica-bearing Hydrothermal Fluids

Quartz crystals are the state mineral of Arkansas, although surprisingly both their age and emplacement are poorly understood. H. D. Miser, a native Arkansan whose entire geological career was with the U.S. Geological Survey, had a life-long interest in quartz crystals and examined their origin and occurrence in detail, publishing several papers on the subject (e.g. Miser 1959). The greatest concentration of quartz crystals is in the Ouachita Mountains, where Miser was able to define the northern and southern limits of the "quartz belt" (Figure 5). Strata representing the upper Cambrian through middle Pennsylvanian are all cross-cut by quartz veins. Unfortunately, the mineral quartz cannot be dated by standard isotopic methods. There are intrusions, all Lower Cretaceous, within the Ouachita Mountains, as well as on the adjacent coastal plain. Interestingly, these intrusions are either felsic (Granite Mountain and the Bauxite region) or ultra-mafic (Magnetic Cove, Potash-Sulfur Springs, Murfreesboro), and none contain quartz crystals (Miser 1959).

The silica-bearing, hydrothermal fluids that have left a record in the upper Boone and Elsey Formations of Arkansas and Missouri may possibly be related to the mineralization of the Mississippi Valley-Type (MVT) ore deposits found within the Tri-State Lead-Zinc Mining District of northern Arkansas, southwestern Missouri, and northeastern Oklahoma.

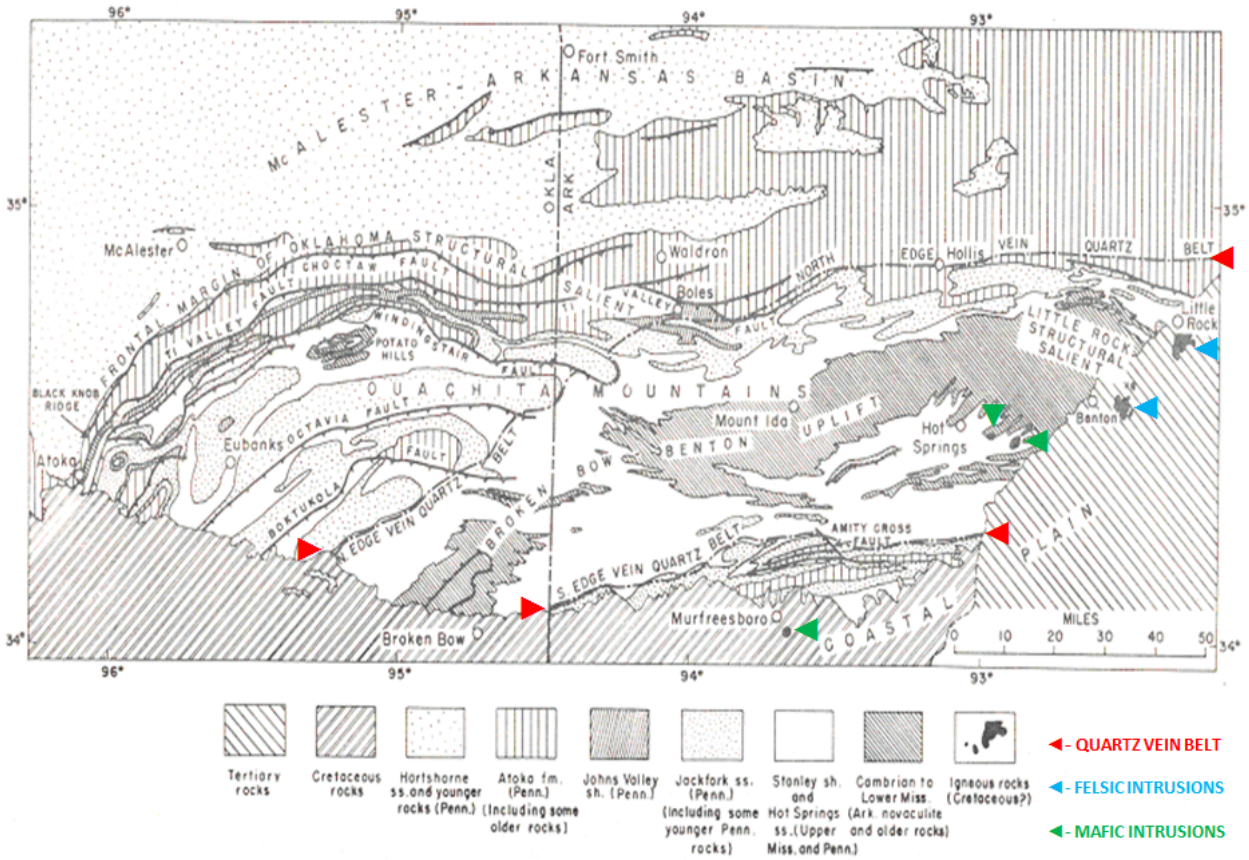

Figure 5. Map of the Ouachita Mountains showing the limits of the "quartz belt" and the felsic and ultramafic intrusions (Miser 1959).

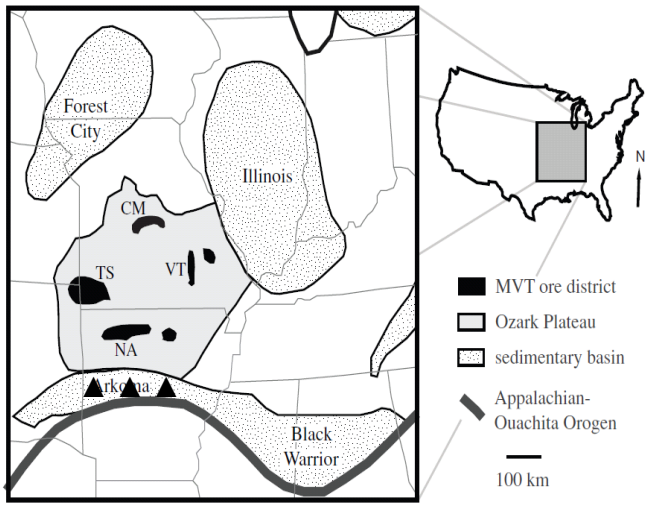

Figure 6 – Relationship and proximity of the MVT ore district to the Ouachita Mountains (arrows denote presumed delivery direction of silica-bearing fluids) (Wenz *et al*. 2012).

As can be seen readily in Figure 6, the alignment of the MVT ore deposits is directly north of the Ouachita flexure, and could have been emplaced by lateral secretion generated by the Ouachita Orogeny in the Late Pennsylvanian.

Conclusions

The tripolitic chert is a replacement of an interval within the base of the upper Boone Formation in Arkansas and Oklahoma, and equivalent to the Elsey Formation in Missouri. The movement of silica-rich, hydrothermal fluids acted like a confined aquifer system bound by impermeable intervals at top of the Lower Boone = Reeds Spring Formation, and the base of the Upper Boone = Burlington-Keokuk Formation. The first hydrothermal event silicified the basal upper Boone = Elsey Formation, while the second hydrothermal event produced terminated and doubly terminated quartz crystals and druse in the voids left in the tripolitic chert. This hydrothermal event may have produced the MVT lead-zinc deposits in northeastern Oklahoma and southwestern Missouri. Although the timing of the series of hydrothermal events is unknown, they may reflect lateral secretion produced by the Ouachita Orogeny in the Late Pennsylvanian.

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Sequence Stratigraphic and Tectono-Stratigraphic Successions, Ozark Shelf, Tri-State Region, Southern Midcontinent

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Running Title: Tectono-Stratigraphy of Ozark Shelf, Southern Midcontinent

Abstract

The southern Ozark region, Arkansas, Missouri, and Oklahoma occupies the southern border of the North American craton. Its sedimentary succession preserves a complete Wilson Cycle reflecting the Late Precambrian-Cambrian rifting of Rodinia into the Laurussian and Gondwanan landmasses that opened the Iapetus Ocean Basin during the Late Cambrian-Middle Mississippian. The basin was closed during the Late Mississippian-Middle Pennsylvanian by the collision of Laurussia with Gondwana. During the Late Cambrian through the Middle Pennsylvanian, the Ozark Shelf, including its gently sloping, Northern Arkansas Structural Platform (NASP) and adjacent ramp, records both transgression and regression by epeiric seas as well as regional tectonism that can be recognized as five Tectono-Stratigraphic Successions (TS) and correlated readily with the Sloss Cratonic Sequences. The TS record comprises at least 33 named formations with a potential thickness >2926m (9600ft). However, both eustatic and tectonic sea-level rise and fall also produced regional surfaces of erosion that punctuated deposition, and the preserved thickness on the NASP is significantly less. The five distinct, but related, Tectono-stratigraphic Successions in the Paleozoic record are (TS1) Late Precambrian-Middle Cambrian, (TS2) Upper Cambrian-lowest Ordovician, (TS3) Lower Ordovician-Lower Devonian, (TS4) Middle Devonian-Upper Mississippian, and (TS5) Lower-Middle Pennsylvanian. TS1, a pre-Late Sauk Sequence, is the least well-known succession, consisting of emplaced igneous and low-ranked metasedimentary bodies and pre-Lamotte sedimentary rocks. TS2, Late Sauk Sequence, is potentially >937m (3075ft) of dolomites and sandstones. TS3, Tippecanoe Sequence, is the penultimate thickest interval, possibly >1257m (4125ft) of dolomites, limestones, shales, and supermature sandstones. TS4, Kaskaskia Sequence, measures at least 736m (2416ft). The final TS5, Lower Absaroka Sequence of first cycle sandstones with variable amounts of mrfs, and shales is the thickest interval, >1267m (4160ft) and may exceed 7620m (25,000ft) in the adjacent Arkoma Basin.

Relationship of Sequence Stratigraphy and Tectonostratigraphy

Depending on their setting, lithostratigraphic successions may reflect two independent, but potentially simultaneous processes: 1) *eustasy* – change in the total volume of global seawater, producing a stratigraphic sequence record, and 2) *tectonism* – change in elevation of earth's crust; uplift or subsidence, providing or reducing accommodation space. Recognition of the effects of these two processes on the geologic record provides the basis for its tectono-stratigraphic divisions.

Sequence Stratigraphy – Although thought to be a relatively new concept, the basic concepts and foundation of sequence stratigraphy were already laid in the 19th century, and developed further through the first-half of the 20th century. Most of the modern understanding of sequence stratigraphy has evolved from the concepts of cratonic sequences published by L. L. Sloss (1963). He recognized that the stratigraphic record of the North American craton (late Precambrian to the present) was punctuated by six, essentially cratonwide, unconformities that defined six successive groupings of strata, or sequences. A complete sequence can be divided into lowstand, transgression, maximum flooding, highstand and regression, although location on the craton influences development of individual stages and some sequences may not be complete (see Sloss 1963, Van Wagoner *et al.* 1988, and Gradstein *et al.* 1998, for further discussion of the development of sequence stratigraphic concepts).

Tectono-stratigraphy – Lithostratigraphic sequences may also record tectonic influences on the depositional succession. This subdiscipline of stratigraphy has been applied since at least 1875, originally describing sequences in large-scale, stacked, thrust sheets (nappes), in tectonically influenced areas, such as thrust belts (Medlicott 1875). More recently, it has been broaden to include the study of any area that exhibits a tectonic imprint on its lithostratigraphy, including the cratonic interiors (e.g., Houseknecht 1986). Change in cratonic elevation is accomplished by seafloor spreading either by underplating continental crustal masses forming domes and mountains or by crustal subsidence from mantle flow away from the craton interior forming basins and rifts. Not all cratonic areas experience active tectonism, and their lithostratigraphic record may only reflect eustatic change. The gently sloping southern Ozark cratonic platform and its adjacent ramp preserve a Paleozoic record of five distinct but related tectono-stratigraphic successions.

Sequence and Tectono-stratigraphic Record, Southern Ozark Region

The Paleozoic sequence record of the southern Ozark region, northern Arkansas, can be divided into 25 cycles comprising the interval from the Late Cambrian through Middle Pennsylvanian (Atokan) periods. This succession is preserved, all or in part, four first-order, seven second-order, and fifteen third-order cycles (Waite 2002; Figure 1). At least three condensed sections and seventeen unconformities punctuate the record (McFarland 2004). The first-order and second-order cycles recognized by Waite (2002) are preserved, but three third-order cycles – between the Middle and Upper Ordovician, the lowermost cycle of the Upper Silurian, and the lowermost cycle of the Middle Devonian strata are missing (Figure 1).

Type - 1 unconformities at the Eminence-Gunter, Roubidoux-Jefferson City, Everton-St Peter, Lafferty-Penters, Penters-Clifty, Clifty-Sylamore, Chattanooga-St. Joe (Bachelor), Boone-Batesville, Pitkin-Hale and Morrowan-Atoka contacts (McFarland 2004) in northern Arkansas correspond to Waite's third-order unconformity surfaces. In contrast, predicted third-order unconformities at the Upper Ordovician-Lower Silurian and Lower-Middle Silurian contacts (Waite 2002) fall within continuously deposited successions.

Composite Thicknesses for the Paleozoic Record, Southern Ozark Region

Thickness data for this study were compiled from published water and geophysical well logs (Huffman 1951; Sheldon 1954; Howe and Koenig 1961; McKnight and Fischer 1970; Johnson *et al*. 1989; Simms *et al*. 1995; Boyd, 2008; Pasteris 2014). The thinnest and thickest preserved stratigraphic intervals of the southern Ozark region vary from 566-2954m (1860-9692ft) and 452-2052m (1483-6733ft) in the northwest and north-central Arkansas, to 880-2729m (2888-8936 ft) and 998-2371m (3276-7781ft) in the southeast and southwest Missouri, and 42-669m (140-2196ft) and 355-729m (1165-2393ft) northeast and Cherokee Platform, Oklahoma, respectively (Figure 2). The sedimentary succession is thickest in northwest Arkansas, where it peaked at 2954m (9692ft), and thinnest in the northeasternmost Oklahoma area, where only a minimum thickness of 42m (140ft) is documented. The tristate area is carbonate-dominated, representing a maximum of 64% of the total Paleozoic sedimentary record in northeast Oklahoma, 59% in southern Missouri, and 46% in northern Arkansas. The remaining section comprises terrigenous clastic sediment with sandstone contributing approximately 18% and shale about 19% of the interval in Arkansas, where they make their greatest contribution.

Paleozoic Tectono-stratigraphic Divisions and Rates of Sedimentation, Southern Ozark Region

TS1 is poorly known, but the TS2 and TS3 divisions show a general Missouri-Arkansas-Oklahoma thinning trend, the TS4 division thins progressively from Missouri into Arkansas and Oklahoma, while the TS5 division thins into Oklahoma and Missouri from Arkansas. The average rates of deposition in Arkansas for TS3 and TS4 are 0.01 mm/year, and 0.03 mm/year and 0.04 mm/year for TS2 and TS5, respectively. In Missouri, the TS3 and TS4 averaged 0.021 mm/year whereas the average rate of the TS2 and TS5 are 0.04 mm/year and 0.001 mm/year, respectively. In Oklahoma, the rate is 0.002 mm/year for TS3 and TS5, while TS2 and TS4 are 0.01 mm/year and 0.004 mm/year, respectively.

Tectono-Stratigraphy of Ozark Shelf, Southern Midcontinent

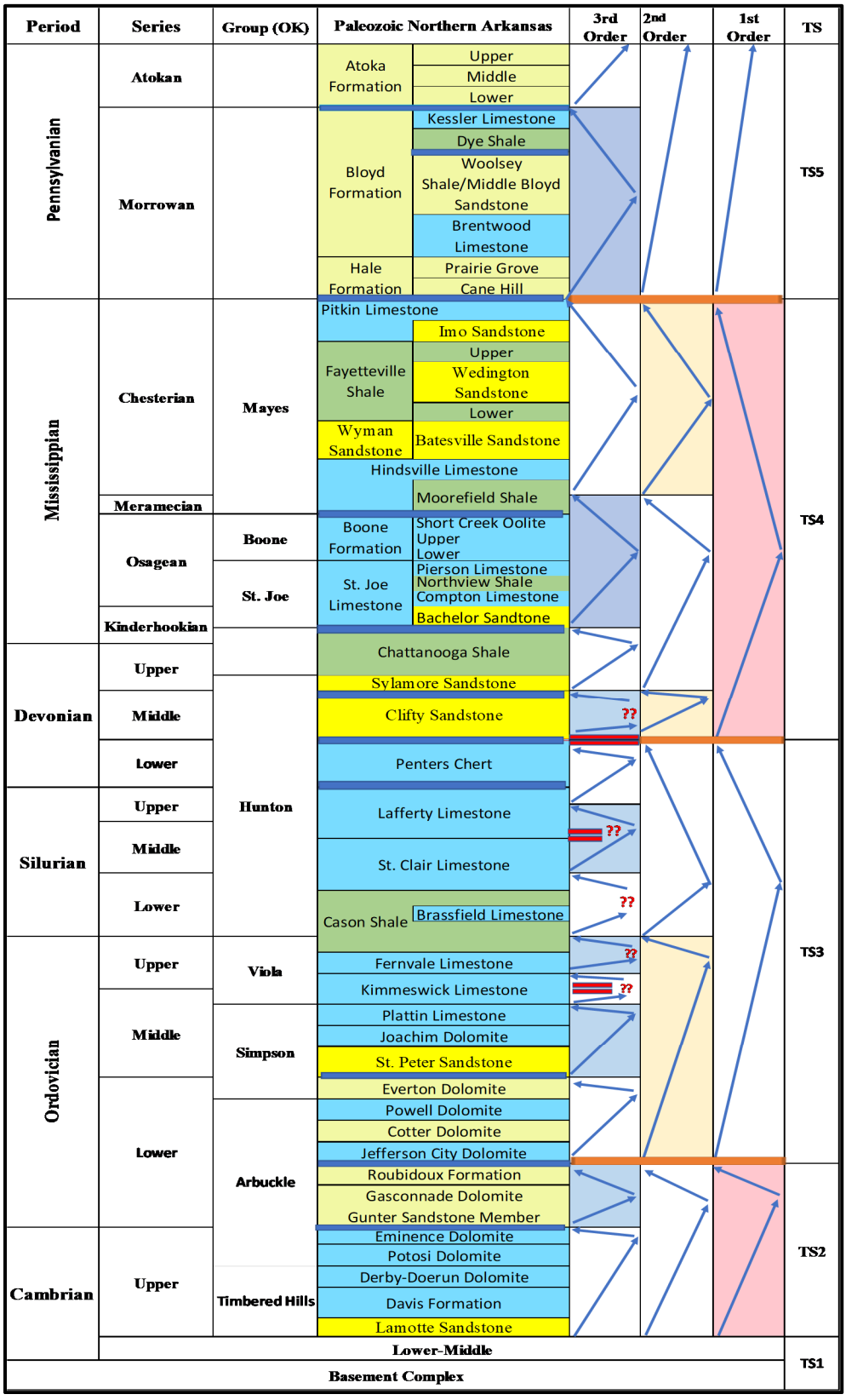

Figure 1 – Chronostratigraphy, Lithostratigraphy, Sequence Stratigraphy, and Tectono-Stratigraphic Assignments, southern Ozark Dome, northern Arkansas, modified from McFarland, 2004 (eustatic cycles from white, 2002) (Colors: Yellow – Sandstone; Green – shale; Blue – Limestone; Tan – mixed lithologies including sandstones).

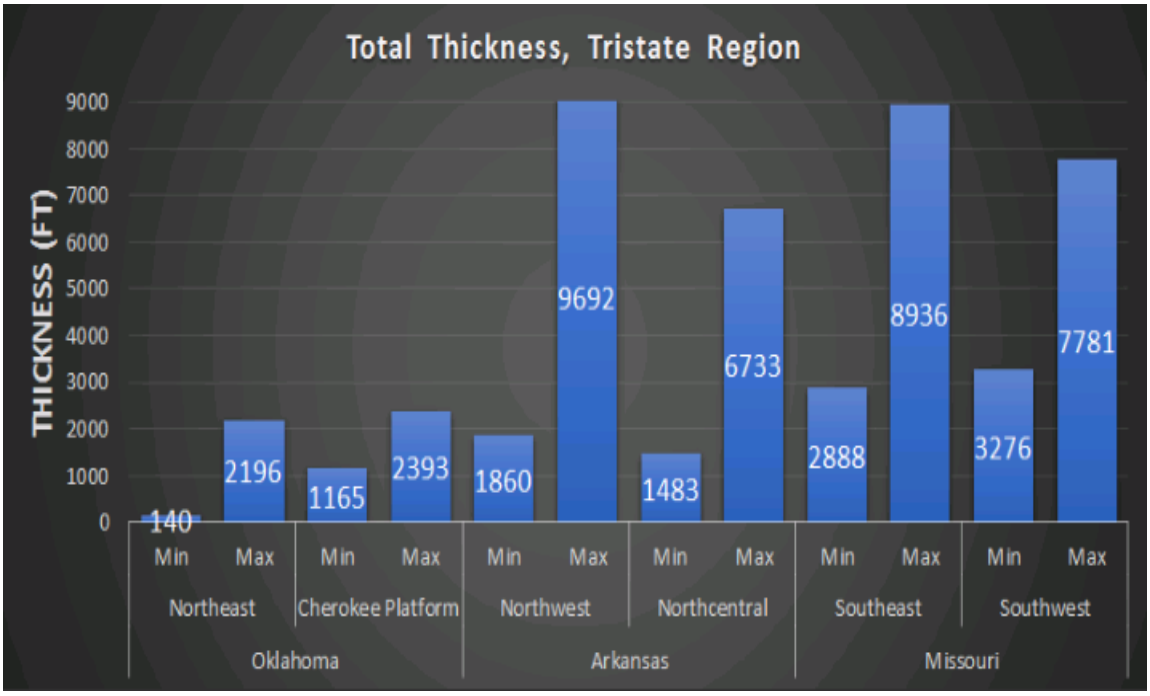

Figure 2 - The composite thicknesses (maximum and minimum) of the Upper Cambrian-Middle Pennsylvanian interval for the Upper Cambrian-Middle Pennsylvanian interval of the southern Ozark Region, Arkansas, Missouri, and Oklahoma. Sources of most thickness data: Arkansas – McFarland 2004; Missouri – Howe and Koenig 1961; Thompson 2001; Oklahoma – Huffman 1958; Johnson *et al*. 1989; see text for full list of sources.

Tectono-stratigraphic Interval 1 (TS1) – Late Precambrian to Middle Cambrian, > 1 Ga.

The poorly known TS1 Interval comprises the basement of igneous, metamorphic, and pre-Lamotte sedimentary rocks, locally present beneath the basal Lamotte-Reagan sandstones. These basement rocks formed between 1.4 billion and 600 million years ago and are exposed in the St. Francois Mountains of southeastern Missouri and the Wichita and Arbuckle Mountains of southern Oklahoma (Johnson *et al*. 1989). In northern Arkansas, the Mowery 1 gas well drilled by Gulf Oil Company in 1968 is situated in Section 14, Township 10N, and Range 32W, Crawford County, and penetrated about 54m (150 ft) of these rocks in northern Arkansas. Houseknecht and Weaverling (1983) documented more than 499m (1640 ft) of a pre-Lamotte section of carbonates and shales in the Reelfoot Rift Basin in northeastern Arkansas. These pre-Lamotte units are the correlative equivalents of the Conasauga (Middle Cambrian) and Rome (Lower Cambrian) Formations of the southern Appalachian Mountains (Houseknecht and Weaverling 1983).

Tectono-stratigraphic Interval 2 (TS2) – Upper Cambrian to earliest Ordovician, ~ 19+ Ma.

The TS2 Interval is a dolomite-dominated interval with a secondary contribution by terrigenous clastic sediment. The component TS2 lithostratigraphic divisions include (ascending order): Lamotte, Bonnetterre-Davis, Derby-Doerun, Potosi, Eminence, Gasconade, Roubidoux, and Jefferson City Formations. TS2 is thickest in the southeast Missouri, where it is approximately 937m (3075ft), and thinnest in the northeastern Oklahoma area, with only a recorded thickness of 73m (240 ft). The minimum and maximum thicknesses across the tristate area range from 215-398m (704-1306ft) and 155-722m (510-2370ft) in the northwest and north-central Arkansas, to 126-709m (416-2327ft) and 423-937m (1390-3075ft) in the southeast and southwest Missouri, and 196-287m (642-940ft) in northeastern Oklahoma. The TS2 interval comprises 35% of the section in northcentral Arkansas, 40% in southwest Missouri, and 39% in the northeastern Oklahoma. Carbonates represent the most abundant lithotype, contributing a maximum of 83% of the total TS2 interval in Arkansas, 78% in southwestern Missouri, and 87% in northeasternmost

Oklahoma. This interval is absent in the northeastern Oklahoma.

Tectono-stratigraphic Interval 3 (TS3) – late Lower Ordovician-Lower Devonian, ~ 85 Ma.

The TS3 Interval is the most well-developed tectono-stratigraphic unit across the tristate region. Its lithostratigraphic divisions include the, ascending order: Jefferson City, Cotter, Powell, Everton, St. Peter, Joachim, Plattin, Kimmeswick, Fernvale, Cason, St. Clair, Lafferty, and Penters Formations. Carbonates dominate the interval, constituting 95% across southern Missouri, 84% in northwest Arkansas, and 97% in northeastern-most Oklahoma, although minor sandstones and shales occur sporadically, and numerous unconformities punctuate TS3. The sandstones of the Cotter, Everton, and St. Peter, Clifty Formations are entirely supermature, orthoquartzite sandstones. The maximum TS3 interval thickness is 722m (2370ft) in north-central Arkansas, 1143m (3751ft) in southeast Missouri, and 233m (764ft) in northeast Oklahoma. The interval contributed up as much as 35% to the northern Arkansas record, 48% in southeast Missouri, and 35% in northeast Oklahoma of the total Paleozoic sediment. Sandstone abundance is greatest in the northcentral Arkansas area, reaching 152m (500ft) thick, 61m (200ft) in southeast Missouri, and 32m (106ft) in northeast Oklahoma. These values correspond to 14% of the maximum TS3 interval in southeast Missouri, 21% in northcentral Arkansas, and 5% in northeast Oklahoma, respectively. Shale is significantly low abundance in the region, except in northeastern Oklahoma, where it reaches only 121m (37ft), yet constitute 16% of the TS3 section in that area. Its greatest thickness is approximately 21m (68ft) in northcentral Arkansas, and the least is 6m (15ft) across southern Missouri.

Tectono-stratigraphic Interval 4 (TS4) – Middle Devonian-middle Upper Mississippian, ~ 70 Ma.

The Clifty, Sylamore, Chattanooga, St. Joe, Boone, Moorefield, Hindsville-Batesville, Wyman, Fayetteville, and Pitkin Formations (ascending order), and their component subdivisions comprise Tectono-stratigraphic Interval 4 (TS4). The section is thickest in the southeast Missouri region, and it thins progressively into northern Arkansas and northeastern Oklahoma. The greatest TS4 thicknesses are 644m (2112ft) in northwest Arkansas, 736m (2416ft) in southeast Missouri, and 312m (1025ft) in northeast Oklahoma. TS4 contributed about 23% of the total lithostratigraphic thickness in the tri-state region. Local irregularities in the TS4 possibly reflect Devonian and Mississippian post-depositional erosion and truncation.

The Clifty Sandstone, Sylamore Sandstone, and Chattanooga Shale make up the Middle-Upper Devonian component of the TS4 section. Their composite thickness is 464m (1522ft) in southeast Missouri, where it is thickest, 41m (134ft) in northwest Arkansas, and 27m (90ft) in Oklahoma. The TS4 Devonian rock is thinnest and missing in most of the northcentral region of Arkansas and the northeastern-most Oklahoma region.

The Mississippian TS4 component is a third-order transgressive-regressive cycle bounded by type 1 unconformities and divided into the St. Joe, Boone, Batesville, Hindsville, Fayetteville, and Pitkin Formations, ascending order. The Mississippian interval is thickest in the northwest Arkansas region, while it progressively thins into Missouri and Oklahoma. Thicknesses vary from 100-603m to 132-470m (329-1978ft to 432-1542ft) in northwestern and northcentral Arkansas, to 114-273m to 103-276m (473-894ft to 339-905ft) in southeastern and southwestern Missouri, and 5-256m to 34-255m (15-845ft to 110-837ft) in northeastern and northeasternmost Oklahoma, respectively. The interval contributes a maximum of 23% in northern Arkansas, 27% in southern Missouri, and 47% in northeast Oklahoma to the entire Paleozoic section in those areas.

The TS4 sandstones account for a maximum of 38% of the total TS4 section in southeast Missouri, 20% in northcentral Arkansas, and 8% in the northeasternmost Oklahoma. The sandstones of the Clifty, Sylamore, and St. Joe Formations (Bachelor Sandstone Member) are supermature quartzarenites. The appearance of first cycle sandstones with a minimum of metamorphic rock fragments (mrfs) in the Upper Mississippian Batesville, Wyman, Wedington, and Imo sandstones distinguish the upper TS4 interval. Shale comprises 40% of the entire TS4 section in northwestern Arkansas, but only 12% in southwest Missouri, and 11% in northeasternmost Oklahoma area.

Tectono-stratigraphic Interval 5 (TS5) - Lower-Middle Pennsylvanian, ~ 16+ Ma.

The TS5 interval constitutes a cyclic succession of sandstone and shale likely derived from the Appalachian and Ouachita regions with minor local carbonate. The Hale Formation (Cane Hill and Prairie

Grove Members), Bloyd Formation (Brentwood Limestone, Woolsey Shale-middle Bloyd Sandstone, Dye Shale-Kessler Limestone), and Atoka Formation (Lower, Middle, and Upper Members) comprise the TS5 group. The TS5 division is the thickest of TS intervals. But component divisions may not be laterally persistent across the region. The greatest thickness is 1268m (4160ft) in northwest Arkansas, 21m (68ft) southeast Missouri, and 124m (407ft) in northeast Oklahoma. The interval contributed a maximum of 43% of the total sedimentary record in northern Arkansas, less than one percent in southern Missouri, and only 19% in northeastern Oklahoma.

As can be seen already in the Upper Mississippian, TS4 first cycle sandstones, metamorphic rock fragments (mrfs) ranging from common to abundant appear in the TS5 sandstone succession. The interval is not present in southwest Missouri, probably because of later erosion, and sporadic to absent across northeastern Oklahoma and southeastern Missouri for the same reason. Local anomalies in the TS5 thickness are the result of both Morrowan and Atokan erosion, which removed some of the pre-Pennsylvanian intervals in the Ozark region and produced the Woolsey/middle Bloyd Sandstone-Dye Shale, and Morrowan-Atokan regional unconformities. Consequently, TS5 strata onlap older rocks toward the Ozark core and progressively thicken to the south. Before TS5 deposition, a Middle-Upper Mississippian uplift exposed the area to erosion and significant karst development before subsequent Pennsylvanian submergence and deposition of the Pennsylvanian TS5 Hale, Bloyd, and Atoka strata. TS5 can be subdivided into three divisions based on an increase in the contribution by metamorphic rock fragments. The terrigenous clastic contribution in the region is highly variable and may be absent in most of the southern Missouri and northeastern Oklahoma areas, while it is thicker and more persistent across northern Arkansas.

Conclusions

The Paleozoic record of the southern Ozark region, northern Arkansas, southern Missouri, and northeastern Oklahoma, accumulated on the gently sloping, cratonic Arkansas Structural Platform (NASP) and its adjacent ramp that experienced transgressive-regressive sequences deposited by epeiric seas, including both first-cycle and reworked terrigenous clastic sediments, as well as blanket shallow-water carbonates. In addition, the region experienced modest and sporadic uplift that affected the component lithologies and their regional distribution. That combination provides the basis for organization of the Paleozoic record into five separate, but related, tectonostratigraphic units (TS1-TS5): (TS1) Late Precambrian-Middle Cambrian, (TS2) Upper Cambrian-lowest Ordovician, (TS3) Lower Ordovician-Lower Devonian, (TS4) Middle Devonian-Upper Mississippian, and (TS5) Lower-Middle Pennsylvanian.

TS1, a pre-Late Sauk Sequence, is the least well-known succession, consisting of emplaced igneous and low-ranked metasedimentary bodies, and pre-Lamotte sedimentary rocks. TS2, Late Sauk Sequence, is potential >937m (3075ft) of dolomites and sandstones. TS3, Tippecanoe Sequence, is the penultimate thickest interval, possibly >1257m (4125ft) of dolomites, limestones, shales, and supermature sandstones. TS4, Kaskaskia Sequence, measures at least 736m (2416ft) of mixed lithologies. The final TS5, Lower Absaroka Sequence, is the thickest interval, >1267m (4160ft) of first-cycle sandstone, and shale, that may exceed 7620m (25,000ft) in the adjacent Arkoma Basin.

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Restrained Shrinkage of Fly Ash Based Geopolymer Concrete and Analysis of Long Term Shrinkage Prediction Models

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Running Title: Restrained Shrinkage of Geopolymer Concrete and Shrinkage Prediction Model for GPC

Abstract

The research presented in this manuscript describes the procedure to quantify the restrained shrinkage of geopolymer concrete (GPC) using ring specimen. Massive concrete structures are susceptible to shrinkage and thermal cracking. This cracking can increase the concrete permeability and decrease the strength and design life. This test is comprised of evaluating geopolymer concrete of six different mix designs including different activator solution to fly ash ratio subjected to both restrained and free shrinkage. Test results obtained from this experimental setup were plotted along with the available empirical equation to observe the shrinkage strain of GPC and a model was suggested to predict the shrinkage strain of GPC. It was found from this study that along with activator solution to fly ash ratio the final compressive strength of GPC plays an important role on shrinkage strain.

Introduction

In high strength concrete structure and concrete repair, overlay, long span slab, differential drying through the thickness of the large mass cause internal restraint and buildup tensile stress within the material (Palomo *et al*. 1999). Tensile stress in the structure also depends upon the external restraint of the structural element. Time to crack depends not only on the tensile strength of the concrete but also on the tensile creep characteristics of the material (Duxon *et al*. 2007). One of the popular tests to determine the early-age-behavior of concrete under restrained shrinkage is the ring test (Moon and Weiss 2006). When the concrete ring deforms due to shrinkage the steel ring restrains the concrete which causes tensile stress in the specimen. In the ASTM C 1581 the ring provides a high degree of restraint while still allowing sufficient strain in the steel as the concrete shrinks (Ryan *et al*. 2010). Cracking in the ring specimen are assessed from the reading obtained from the strain gages attached to the inner surface of the steel ring. This method provides the strain data which can be converted with suitable mathematical equations to the stress developed in the concrete ring (See *et al*. 2003). An instrumented ring similar to the ASTM C1581 ring was evaluated in this study and used to obtain the restrained shrinkage behavior of six geopolymer concrete mixtures. Dimension of the ring specimens and thickness of the steel and concrete ring was selected according to the standard to follow the empirical equations already developed for stress calculation for restrained ring specimen (Jun *et al*. 2011). Testing and analysis procedure presented in this study illustrates how instrumented ring specimen can provide data on restrained stress and strain of geopolymer concrete. These results provide a basis for comparing the performance of different GPC mixtures under restrained shrinkage in the same environmental condition (Swayze 1942). This study deals with the result from shrinkage tests on the geopolymer concrete mix on both restrained and free shrinkage condition. Test was conducted to see the age of cracking and free shrinkage strain of geopolymer concrete. Data analysis was performed to evaluate the effect of various factors on the shrinkage behavior. Statistical analysis was conducted to establish the relationship between compressive strength at the age when shrinkage test was started and ultimate shrinkage strain. A theoretical model was emphasized and compared with existing empirical models to see the effectiveness of the best prediction equation for GPC.

Materials and mix design

Concrete mixtures were selected with different activator solution to fly ash ratio and for different target strength of the hardened concrete. Variables were selected to see the effect of activator solution to fly ash ratio on the shrinkage strain of geopolymer concrete. Compressive strength of the concrete varied in ranges between 25 MPa to 55 MPa. Samples were prepared without using any shrinkage reducing admixtures

(Guneyisi *et al*. 2010). Total amount of coarse and fine aggregate was constant for different mix design to see the effect of geopolymerization on the short and long term properties of GPC. All the mixes showed more than an 8 inch slump and the air content was below 4%. Concrete rings were kept on a vibrating table for 30 seconds to remove any entrapped air bubble inside it. A total of 6 different GPC and 1 ordinary portland cement (OPC) concrete mixture were evaluated. OPC mix was used as a control sample to monitor the shrinkage property from the testing and adjustment of the test setup.

Mix Proportion of Concrete

Concrete mixtures were selected from the specific strength range using the particular mix design of the activator solution and fly ash type. Strength of the concrete was controlled by the variation in activator solution to fly ash ratio (AS/FA). Class F fly ash was used for the design of concrete cylinders. Four different mix designs were produced by varying the AS/FA. Mix design was selected from the preliminary test. The detailed mix proportion for this group of specimens is presented in Table 1.

The second set of mix design was prepared to observe the effect of the extent of geopolymerization. In this test program, the aggregate to fly ash ratio was kept constant. Minimum compressive strength was attained using N silicate and 10M sodium hydroxide solution, and high strength was achieved using D silicate and 14M sodium hydroxide solution (Table 2). Activator solution to fly-ash ratio was 0.35 for both mix design.

A control mix of OPC to compare the results with the GPC was designed following the ACI guideline. OPC mix design was prepared to see the propagation of cracks and to use as a reference. Mix proportion was selected to get a hardened concrete with nominal strength of 55 MPa. Water cement ratio for this mix was 0.3.

Table 1. Mix design of GPC with the variation in AS/FA ratio.

Raw Material	Mix Design for different activator solution to fly ash ratio (kg/m^3)			
	0.35	0.45	0.55	0.65
NaOH (12M)	78.3	100.9	122.8	145.4
Silicate (N)	117.5	151.3	184.6	218.4
Fly Ash	559.6	559.6	559.6	559.6
River Sand	719.8	719.8	719.8	719.8
Pea Gravel	868.8	868.8	868.8	868.8

Table 2. Mix design of GPC with the variation in compressive strength.

Mix design for 25 MPa GPC (kg/m^3)		Mix design for 50 MPa GPC (kg/m^3)	
NaOH (10M)	78.3	NaOH (14M)	78.3
Silicate (N)	117.5	Silicate (D)	117.5
Fly Ash	597.5	Fly Ash	597.5
River Sand	612.4	River Sand	612.4
Pea Gravel	881.5	Pea Gravel	881.5

Table 3. Mix design of high strength OPC.

Working mix design in (kg/m^3)	
Cement (type-I)	692.5
Water	207.5
River sand	630.2
Pea gravel	868.5

The particular mix design in Table 3 was used to make a set of samples to find the strength gain over time and other mechanical properties. OPC samples were prepared and stored according to ASTM C31.

Test Method and Sample Preparation

The shrinkage test apparatus was prepared following ASTM C1581. The mold was prepared with a metal pipe section as the inner ring and a PVC two-part outer ring. Strain gages were attached to the inner surface of the metal ring to calculate the shrinkage strain caused by the drying of concrete. The data acquisition system was used to calculate the deformation occurred in the strain gage and the stress in concrete was also analyzed from this data. Ring specimens are more commonly used because of the benefits that those can easily be cast and the end effects are removed providing an axi-symmetric geometry (Kovler 1994). If the thickness of the steel is too large, deformation cannot be detected from the experiment. Such test setups provide qualitative evaluations, but do not establish a simple procedure to routinely quantify the restrained characteristics of the material (Grzbowski and Shah 1989). Strain at the inner surface of the steel ring is measured by the foil strain gage, which provides an accurate assessment of the time to crack. Cracking of the test specimens are indicated by a sudden decrease in compressive strain in the steel ring. The measured strain provides the basis for quantifying the restrained shrinkage behavior of the concrete specimen. Strain gages were placed at mid-height of the steel annulus, where the average strain is measured. Thickness of the

concrete wall was maintained at 1.5 inch for all specimens (Kwesi *et al*. 2014). The steel ring for the inner part of the mold was prepared from the steel pipe section of the standard size. The dimension of the steel ring was selected following ASTM C1581. Thickness of the steel ring was 0.5 inch and the inside diameter of the ring was 12 inch (30.48 cm). Steel pipe was cut according to the specified height 6 inch (150 cm) given in the standard. The edge of the ring was ground with fine sand paper. The inner and outer surfaces of the steel ring were cleaned using the sand blasting apparatus to remove any oil and grease. The rings made from the pipe section were further prepared to install the foil strain gage at the inner surface. Two strain gages were attached to the surface 180º apart. Data collection from the acquisition system was stopped when the crack formed at the outer surface of the concrete propagated to the inner ring, and there was no change in the reading obtained from the strain gages. The rate of shrinkage can change due to temperature and relative humidity. It is very important to keep concrete specimen inside a controlled environment to measure the shrinkage accurately. For this test an environment chamber with a dimension of 30ft x 15ft x 8ft was made with thick insulated aluminum wall.

The environmental control chamber kept the specimens at controlled temperature and humidity without too much stress on the mechanical devices. There was an arrangement to read the actual temperature and humidity by the digital panel from outside the chamber. For this experiment the environment chamber was kept at a constant temperature of 73±3º F and a relative humidity of 50±4% (Qiao *et al*. 2012).

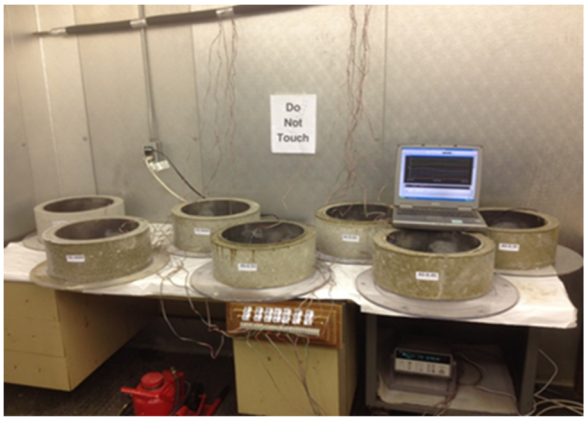

Figure 1. Test setup for strain measurement

Strain Data Calculation

Each sample was equipped with two strain gages. In order to record the strain for three months of testing, the data acquisition system is essential. Data logger used was manufactured by Hewlett Packard with 96 channels for strain readings. This arrangement allowed multiple data to be recorded at the same time. Every three days data was collected from the data logger to a computer. Data obtained from the data acquisition system was processed through the data logger software. Regular observations were made to see whether there is a trend of cracking in any of the ring specimen. Cracking strain capacity on the other hand was also determined by the elastic modulus test and splitting tensile strength test (Temuujin *et al*. 2009). Drying shrinkage strain was calculated considering the elastic and tensile creep strain in the concrete and balanced with the elastic contraction strain in the steel (Shah and Weiss 2006).

$$\varepsilon_{sh}(t) = \varepsilon_e(t) + \varepsilon_{cp}(t) + \varepsilon_{st}(t) \qquad (1)$$

Where $\varepsilon_{sh}(t)$ is the shrinkage strain, $\varepsilon_e(t)$ is elastic concrete strain, $\varepsilon_{cp}(t)$ is tensile creep strain and $\varepsilon_{st}(t)$ is the elastic steel strain at time t. Tensile stress in the concrete $\sigma_t(t)$ at time t is obtained from the following equation

$$\sigma_t(t) = \frac{E_{st} r_{ic} w_{st}}{r_{is} w_c} \varepsilon_{st}(t) \qquad (2)$$

Here E_{st} is the modulus of elasticity of steel. w_{st} and w_c are the wall thickness of the steel and concrete and r_{ic} and r_{is} are the internal radius of the concrete and steel respectively.

Theory

In 1982, the American Concrete Institute (ACI) recommended the procedure for the prediction of creep and shrinkage in its ACI-209R-82 code provisions (ACI 1982). The main inputs for shrinkage prediction are relative humidity, specimen size, curing period and age of loading. This model predicts the shrinkage strain. Correction factors are applied if the conditions are different from the ideal condition stated in the standard (Hardjito *et al*. 2004). This model can be applied to different kinds of concrete and is very simple to apply. The ACI-209R-82 code recommends the following expressions for shrinkage:

$$\varepsilon_{sh}(t, t_c) = \frac{t - t_c}{T_c + (t - t_c)} \varepsilon_{shu} \qquad (3)$$

According to CEB-FIP code proposed in 1990 and

is restricted to ordinary structural concrete. This model is based on the work of Muller and Hillsdorf (Hossain *et al*. 2003). The main input factors for the prediction of shrinkage are ultimate compressive strength, volume to surface ratio, age of curing, age of loading, and relative humidity. Unless special provisions are given, the model is valid for ordinary structural concrete having a compressive strength of 3000 psi (20 MPa) to 15000 psi (100 MPa), mean relative humidity 40-100% and mean temperature 5°C-30°C. Shrinkage strain was calculated from

$$\varepsilon_{sh}(t,t_c) = \left[160 + 10\beta_{sc}\left(9 - 0.1 f_{cm}\right)\right] \times 10^{-6}\beta_{RH}\sqrt{\frac{\{t - t_c\}}{\left\{350\left(\frac{2A_c}{100\mu}\right)^2 + (t - t_c)\right\}}} \quad (4)$$

B3 model as proposed by Bazant and Baweja (1995). It was developed at Northwestern University and is based on the statistical analysis of shrinkage data in a computerized data bank involving about 15,000 data points and about 100 test series. The latest B3 model considers more parameters than other prediction models (Bazant and Baweza 1995). The following parameters are used: a) relative humidity, b) exposure of concrete specimen to temperature prior to drying, c) size, d) cement type, e) coarse and fine aggregate, f) concrete density, g) concrete age, h) specimen ultimate strength. This model is predicted for w/c ratio of 0.30 to 0.85 and strength 2500 psi (17 MPa) to 10000 psi (65 MPa), a/c ratio 2.5-13.5 and cement content 160-720 kg/m^3. The mean shrinkage strain in the cross section is expressed as:

$$\varepsilon_{sh}(t,t_c) = -\varepsilon_{shu}\kappa_h S(t - t_c)$$

$$\varepsilon_{shu} = \alpha_1\alpha_2\left(0.091\, w^{2.1}\left[\left(f_{cm}\right)^{-0.28} + (270)\right]\right) \times\left(\frac{E_c(7 + 600\, t)}{E_c(t_c + \tau_{sh})}\right) \quad (5)$$

Sakata proposed this model for creep and shrinkage on concrete by a statistical method on the basis of experimental data. The equation can estimate the concrete creep and shrinkage strain (Sakata 1993). These prediction equations of shrinkage were adopted as the Japanese standard methods by the Japan Society of Civil Engineers (JSCE) in the revised standard Specification for Design of Construction and Concrete Structure published in 1996.

$$\varepsilon_{sht} = 0.177c + 121(w/c) - 16\log f'(t_0) - 31.4 \quad (6)$$

Gardner and Lockman (2001) proposed the GL 2000 model following the factors: a) relative humidity, b) average compressive strength, c) concrete member size, d) water to cement ratio, e) cement type, f) modulus of elasticity of concrete at the age of loading, g) concrete age at drying and h) concrete age at loading. This model is calibrated for compressive strength in the range of 2320 psi (16 MPa) to 11890 psi (80 MPa), with volume to surface ratio larger than 0.76, and w/c ratio between 0.40 to 0.60 (Gardner and Lockman 2001). The creep coefficient in this model is dependent on volume to surface ratio, age of drying, age of concrete at loading, and relative humidity. Following equations are used to calculate the creep compliance.

$$\varepsilon_{sh}(t,t_c) = \varepsilon_{shu}(1 - 1.18h^4) \times\sqrt{\left[\frac{t - t_c}{t - t_c + 0.15(V/S)^2}\right]} \quad (7)$$

Mix designs that survive longer without cracking are considered to perform better than those which crack earlier. The cracking area is also an indication of the performance of a mix. Some of the specimens which may crack early but have small cracks, and may not propagate toward the steel ring. Usually, the ring specimens start cracking from the outer surface near either the top or the bottom, and then the crack continues to move inward toward the ring over time (Bentz *et al*. 1995). The speed at which the crack propagates toward the steel ring depends on the mix design. It is possible that the crack does not propagate fully towards the ring. When the crack reaches the ring, it causes a release of compressive stress upon the steel ring. Shrinkage strain values for different mix designs were calculated using the empirical equations and test data obtained from the data acquisition system were compared. In this study ACI, Bazant B3, CEB, GL2000, and Sakata models were evaluated on their effectiveness and accuracy in predicting the shrinkage strain of the different GPC mixes. Tensile creep parameters and restrained shrinkage strain calculation was performed using the free shrinkage strain, steel ring stain, modulus of elasticity and flexural tensile strength of GPC. Empirical equations given in ACI 209 were used to calculate the predicted strain at time t. Given the elastic

strain at cracking, an analysis based on free shrinkage strain alone without considering the tensile creep will give cracking of the concrete much earlier than the actual cracking days. Thus, the tensile creep significantly increased the time to cracking of all concrete mixtures. As expected the lower activator solution to fly ash ratio for GPC mixtures yield longer times to cracking (Jensen and Hansen 2001). This difference is explained by considering the magnitude of tensile creep effect on the cracking resistance. The larger magnitude of tensile creep coefficient of high strength low activator solution to fly ash ratio mixtures also corresponds to the longer days to cracking. This result is also linked to the higher geopolymeric reaction in high strength GPC. The tensile creep coefficients under restrained shrinkage are smaller than the coefficient under free shrinkage and fixed stress (Zuanfeng *et al.* 2011). A lower tensile creep under a state of increasing stress occurs when the specimen is restrained.

Results and Discussion

The cracking behavior of a particular mix is very much dependent upon the liquid content of the mix. The environmental factors such as humidity and temperature that changes the shrinkage behavior were kept constant for all samples so the evaporation effect was neglected. In this study, one of the reason behind the early age cracking was found to be the liquid content of the concrete mix. Non-structural causes were: plastic shrinkage, thermal deformation and autogenous shrinkage. Plastic shrinkage occurs because of differential settlement and excessive evaporation of water from the concrete surface. Thermal shrinkage is largely due to considerable heat generated from the chemical reaction. Autogenous shrinkage is caused due to reduction of volume and self-desiccation of internal pores. Free shrinkage test results were obtained from the prism specimen using the length comparator. The results obtained from the test are shown in Figure 2. Among the models used to predict the free shrinkage of the concrete, the Sakata model was very close to the experimental data observed for various GPC samples. From the test results, it was observed that the water content has a significant effect on the drying shrinkage of the GPC. For a mix design with 0.35 AS/FA ratio, it took 86 days and with 0.65 AS/FA ratio, the concrete ring was cracked in only 42 days. GPC with higher strength took more time to form the surface crack. The high strength of the GPC prevents the tensile crack formation. Test result obtained from 4000 psi and 8000

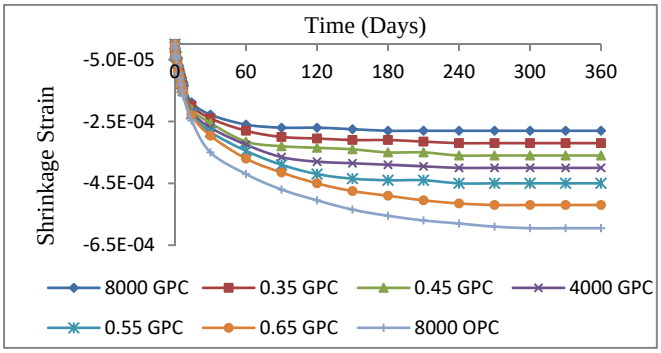

Figure 2. Free shrinkage strain.

psi GPC reflect the hypothesis that geopolymer with high strength has less drying shrinkage than that with lower compressive strength and lower polymerization reaction. Test result obtained from this study puts forth a table with this coefficient for the shrinkage measurement of the GPC with a different activator solution to fly ash ratio. The sand to aggregate ratio had little effect on the mechanical properties or the cracking potential of the mixes.

From compressive strength test results, it was found that OPC had 28 days strength of 55 MPa. GPC sample designed for this similar strength acquired this after 24 hours of heat curing. Figure 2 shows that the first two weeks strain rate is steep for all specimens while there is a change in strain rate at the end of two weeks. After 120 days GPC sample with different AS/FA ratio reached a steady state. At the end of one-year strain in the OPC sample is 200% more than the strain in the GPC sample having the same liquid content at the beginning (35%). The reason behind this can be associated with the formation of a dense polymer matrix that leaves little space for shrinkage in GPC. It can be observed from the graph that the maximum strain from the GPC sample was more than 500 micro strain (for 0.65 AS/FA) and the minimum was around 200 micro strain (for 0.35 AS/FA). This is important data to use in combination with the total strain to find the basic shrinkage for the corresponding mix design of the GPC.

A characteristic comparison plot is shown in Figure 3. All control data are available upon request. It can be observed that ACI model overestimated the shrinkage strain. Strain obtained from the SAKATA model successfully predicted and was very close to the experimental data. Same phenomena were observed for the other samples with different activator solution to fly-ash ratio. This is why the SAKATA model is recommended to predict the shrinkage strain of GPC.

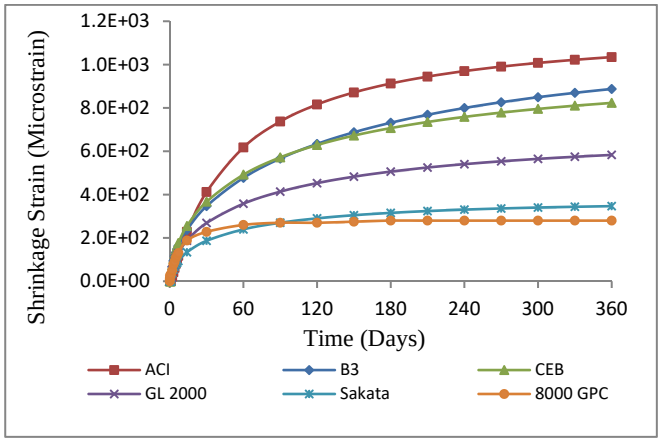

Figure 3. Empirical and experimental data plot

Conclusion

In this paper free and restrained shrinkage of geopolymer concrete was measured at a constant temperature and humidity. The effect of activator solution to fly ash ratio and final compressive strength of GPC was observed on the shrinkage behavior over time. It has been observed that the free shrinkage strain of GPC is less than the data predicted by the empirical equation most of the cases. Each of the mixes had an elastic modulus in the range of about 5000 Ksi (34 GPa) and a tensile strength in the range of 650 Psi (4.5 MPa). Every mix in the AS/FA group cracked around 90 days or stopped putting any compression on the inner ring. The free shrinkage at day 90 for each mix was in the range of 350 to 450 micro strain. Tensile stress generated by restrained shrinkage of the concrete are significant in the first week after casting and lead to a fracture of the material. The role of the tensile creep in the relaxing shrinkage stress is substantial and reduces the stress. The SAKATA model had the closest agreement with the experimental data. The overall comparison with the available models showed the proposed model in this study has the closest correlation with the experimental data.

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Bioassessment of Four Karst Springs at Hobbs State Park – Conservation Area with a Focus on Diving Beetle (Dytiscidae: Hydroporinae) Species of Concern

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Running Title: Bioassessment of Springs at Hobbs State Park Conservation Area

Abstract

Four springs were surveyed at Hobbs State Park-Conservation Area to provide an initial bioassessment and to determine occurrences of two endemic predaceous diving beetles of concern, *Heterosternuta sulphuria* and *Sanfilippodytes* sp. Habitat in the four spring runs were dominated by bedrock and gravel substrate with heavy accumulations of leaf litter. Thirty-three taxa representing 11 orders were collected from the four springs. Non-insect taxa included Oligochaeta, Physidae, and Isopoda, and predominant insect orders included Ephemeroptera, Coleoptera, Diptera, and Trichoptera. The total number of taxa across springs ranged from seven to 19, with total abundances ranging from 39 to 86 individuals. No individual taxon occurred across all four springs. Percent tolerant organisms and the Hilsenhoff Biotic Index showed that spring communities were dominated by taxa tolerant to organic pollution, likely because of low flows and heavy accumulations of leaves. Predators were the dominant functional group followed by shredders. The endemic, predaceous diving beetle *Heterosternuta sulphuria* was collected from two springs and *Sanfilippodytes* sp. was collected from three springs. One spring contained the largest number of *Sanfilippodytes* sp. individuals recorded among all other aquatic habitats surveyed to date. Findings highlight the importance of spring systems at Hobbs State Park Conservation Area for endemic-species conservation, while information on the invertebrate community provides a baseline for future monitoring and comparison.

Introduction

The karst geology of the Ozark Mountains of the U.S. Interior Highlands is the foundation of a landscape thriving with surface and subsurface aquatic habitats. Allen (1990) noted that the Ozarks likely have been a permanent fixture on the landscape for over 300 million years, providing island refugia for organisms when the region was surrounded by ancient seas. Numerous endemic organisms occur in the region and many of these are aquatic species occurring in surface and subterranean aquatic habitats (Robison *et al*. 2008). Perennial aquatic habitats such as freshwater springs are important components of these systems, providing both hydrologic connectivity and flow permanence to support populations when other water sources become unavailable as a result of stream drying (Roughley and Larson 1991; Erman and Erman 1995; Williams and Williams 1998; Smith and Wood 2002). Understanding habitat conditions and biota of these systems is essential for conservation and long-term monitoring.

Aquatic invertebrates contribute to the natural processes of freshwater systems, including nutrient cycling, decomposition, regulation of primary production rates and water clarity (Wallace and Webster 1996). However, aquatic invertebrate communities are exposed to major environmental stressors that threaten biodiversity and these natural processes (Strayer 2006). Some groups of aquatic insects that depend on high-quality habitats in upland streams, such as numerous stoneflies, are considered highly imperiled in the U.S. (DeWalt *et al.* 2005).

Freshwater bioassessment provides a means to summarize the conditions and evaluate the health of aquatic communities in relation to reference (i.e. least-affected) conditions or known responses to environmental stressors (Resh and Jackson 1993). Typically, *metrics* are calculated that summarize benthic macroinvertebrate communities (e.g. species richness or percent predators). Assemblage metrics can be used to both document initial conditions and to monitor potential changes in conditions over time. In mountainous regions where small, wadeable streams

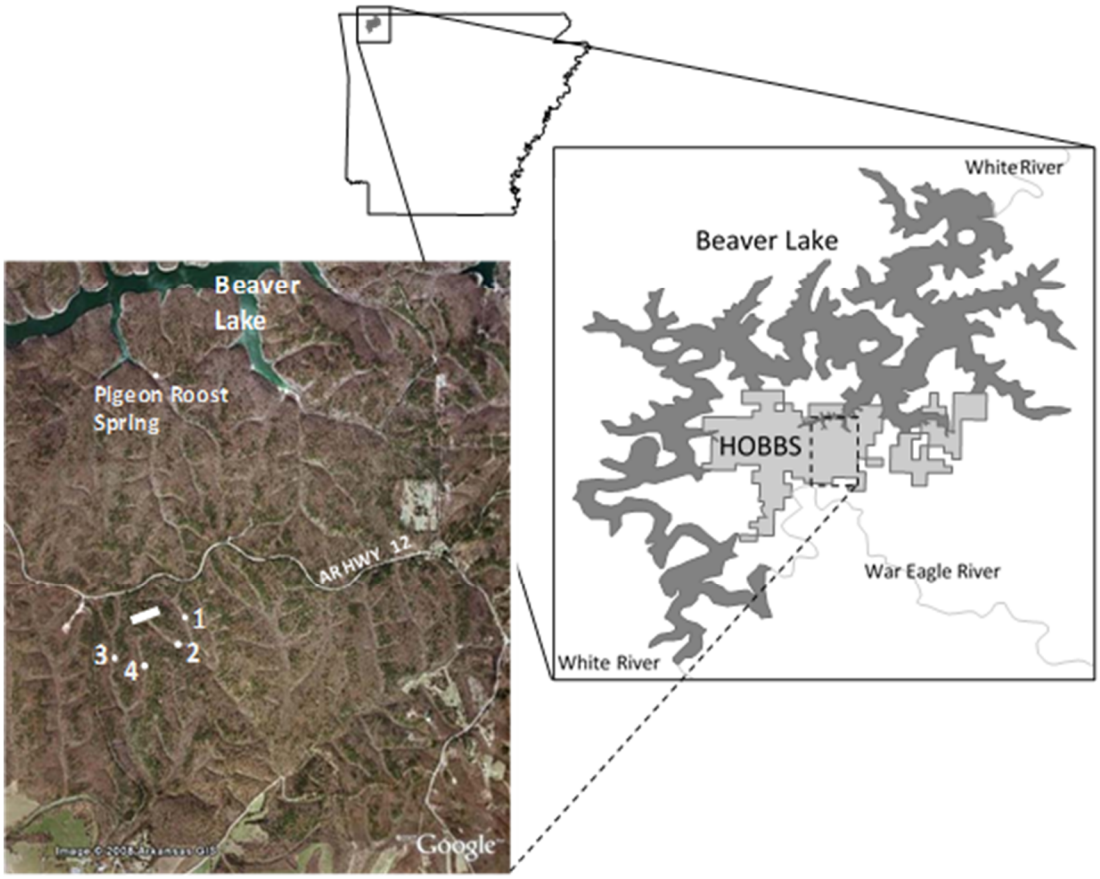

Figure 1. Hobbs State Park-Conservation Area in northwest Arkansas showing the location of the four springs where bioassessments were conducted (springs 1 - 4) and the spring where *H. sulphuria* was first collected at HOBBS, at the terminus of Pigeon Roost trail. Location of the visitors center is shown as a white rectangle.

dominate as a result of the dendritic pattern of stream networks, bioassessment is an effective tool for concurrently surveying multiple streams by both researchers and volunteer-monitoring groups (Engel and Voshell 2002).

Hobbs State Park Conservation Area (HOBBS) comprises 4,874 ha within the Springfield Plateau, a subdivision of the Ozark Highlands Ecoregion (Arkansas Department of Parks and Tourism 2008, Woods *et al*. 2004) (Fig. 1). The region is underlain with cherty limestone from the Mississippian Boone formation. The limestone is highly soluble and has eroded over time, forming many karst features including underground drainage, caves, springs, springbrooks, seeps, disappearing streams, and sinkholes. The moderate topographic relief consists primarily of limestone glades and narrow ridges divided by steep hollows that are vegetated by an upland forest of pine, oak and hickory (Woods *et al*. 2004). One-third of the HOBBS perimeter is in contact with Beaver Lake, the region's 11,480-ha primary source of drinking water. In 1979 land was acquired and legislation enacted to create HOBBS with the mission "to provide enriching educational and recreational experiences in harmony with resource stewardship" (Friends of Hobbs State Park-Conservation Area 2004). HOBBS is jointly managed by the Arkansas Game and Fish Commission, Arkansas State Parks, and the Arkansas Natural Heritage Commission.

During a previous survey of a single spring at HOBBS, we documented the occurrence of two diving beetles, the Ozark-endemic *Heterosternuta sulphuria* Matta and Wolfe and another diving beetle in the genus *Sanfilippodytes* Franciscolo (Longing and Haggard 2009). *Heterosternuta sulphuria* is a species of concern in the Arkansas Wildlife Action Plan (Anderson 2006). Additional occurrences of these species have been further documented from regional streams and springs in the region (Longing *et al*. 2013). Following this initial survey, we selected four additional springs to both document additional occurrences of these diving beetle species of concern and to provide a baseline for further

monitoring. Here, we report those findings to support strategies for conservation aimed at protecting these unique aquatic habitats.

Materials and Methods

Information on springs of HOBBS was reviewed in order to select perennial springs, or springs known to maintain at least some surface water over time. Using historical maps provided by HOBBS superintendent M. Clippinger showing the occurrences of perennial springs, we selected four springs located on opposite sides of 3 adjacent ridges and separated by approximately 500 m (Figure 1, springs 1 - 4). Springs emerged in small, narrow valleys and emptied into spring runs of short lengths (25-100 m) that flowed over limestone bedrock and gravel in narrow channels. The valley slopes were heavily wooded, providing mature canopies that shaded springs. All springs were located in proximity to and immediately south of the HOBBS Visitors Center.

Bioassessments of the four springs were conducted in March 2008. At each spring, the sampling reach was marked by measuring 50 m along the bank with a tape, with the upstream end of the reach located within 5-10 m below the observed spring source or the point of water accumulation. At spring four, the length of the reach did not extend to 50 m, therefore only 25 m was surveyed. Along each reach, wetted widths and water depths were recorded at transects spaced 5 m apart and perpendicular to the channel. From a limited number of locations (i.e. where flows and depths were sufficient for flow-meter measurements) we measured flow velocity and estimated discharge (m^3s^{-1}). At the midpoint of each reach, water temperature (°C), pH, electrical conductivity (EC, $\mu S \cdot cm^{-1}$), and dissolved oxygen (DO, $mg \cdot L^{-1}$) were recorded using a portable YSI 85 meter. Reaches were further characterized by recording dominant and sub-dominate substrate along each transect.

Invertebrate sampling was standardized by collecting invertebrates for 1 hr. from all available habitat types within reaches. D-frame nets (350µm) were used to collect invertebrates by either kicking upstream of the net and letting debris flow through the net or by jabbing the net in habitats where flow velocities were low. We collected from up to approximately 50 percent of the total habitat areas within reaches to avoid over-collecting. Invertebrates were picked from the nets in the field and preserved in 70 percent ethanol in glass vials. Invertebrates were identified to the lowest practical taxonomic level, usually genus (Merritt *et al*. 2007), while predaceous diving beetles were identified to the level of genus or species (Larson *et al*. 2000).

Total number of taxa and total number of individuals for each spring were calculated, in addition to assemblage metrics describing the taxonomic, functional feeding group, habit group and pollution-tolerance of the invertebrate assemblage (Resh and Jackson 1993) (Table 3). Tolerance values were assigned to each taxon using values from Merritt *et al*. (2007). We additionally calculated the Hilsenhoff Biotic Index, a metric that summarizes the tolerance of the invertebrate assemblage to organic pollution weighed by the relative abundance of each taxon, using the following formula (Hilsenhoff 1987):

$$HBI = \frac{\sum n_i \mathbf{x} a_i}{N}$$

Where n = number of specimens in taxon *i*, a = tolerance value of taxon *i* and N = total number of specimens in the sample. A higher HBI represents greater tolerance of the assemblage to organic pollution.

Results

Physico-chemical properties varied across the four adjacent springs (Table 1). Mean depth ranged from 2.9 cm at spring 3 to 5.1 cm at spring 2. Discharge rates were very low; for springs 1, 2, and 4 discharge was estimated to be < 0.02 $m^3 \cdot s^{-1}$ (< 0.5 cfs) while spring 3 had a rate > 0.02 $m^3 \cdot s^{-1}$. Habitat substrate was dominated by bedrock, leaf packs, large and small gravel, and with some bryophytes covering bedrock at channel margins. Conductivity for springs 1 and 2 was 71.2 and 58.2$\mu S \cdot cm^{-1}$, respectively. Conductivity was considerably higher for springs 3 and 4 at 140.6 and 152.4$\mu S \cdot cm^{-1}$, respectively. Dissolved oxygen ranged from 5.36$mg \cdot L^{-1}$ at spring 1 to 9.85$mg \cdot L^{-1}$ at spring 3.

Thirty-three taxa representing 11 orders were collected from the 4 springs (Table 2). Three non-insect taxa collected from the springs were Oligochaeta Physidae, and Isopoda. Insect orders collected included Ephemeroptera, Coleoptera, Diptera, Hemiptera, Megaloptera, Odonata, and Plecoptera. Coleoptera and Diptera were the most diverse insect orders with 7 taxa each. Other insect orders containing multiple taxa included Ephemeroptera (3 taxa), Trichoptera (5 taxa), and Megaloptera (2 taxa). Across the 4 springs, insect orders containing only 1 taxon included Plecoptera, Hemiptera, and Odonata.

Table 1. Physico-chemical properties of four springs at Hobbs State Park-Conservation Area.

Variable	Spring 1	Spring 2	Spring 3	Spring 4
GPS coordinates	N 36°17'05.5" W 93°56'06.6"	N 36°16'58.7" W 93°56'10.1"	N 36°16'57.9" W 93°56'29.4"	N 36°16'55.3" W 93°56'21.5"
Dominant substrate	bedrock	large gravel, leaf packs at margins	bedrock and cobble	bedrock
Sub-dominant substrate	leaf packs on margin	bedrock	large gravel and leaf packs	leaf packs and small gravel
Spring-run length (m)	50	50	50	25
Mean depth (cm)	3.2 (± 3.3)	5.1 (± 2.5)	2.9 (± 3.3)	4.3 (± 3.0)
Bank width (m)	0.5	1.2	2.5	0.5
Discharge (m^3s^{-1})	<0 .02	< 0.02	>0 .02	<0 .02
pH	8.45	7.6	8.23	7.9
Temperature (°C)	12.3	12.2	12.5	11.4
Conductivity (μS cm^{-1})	71.2	58.2	140.6	152.4
DO (mg L^{-1})	5.36	8.12	9.85	7.59

Spring 2 was the most biologically diverse with 19 taxa and spring 4 was the least diverse with 7 taxa. The total number of individuals among springs ranged from 39 (spring 1) to 86 (spring 3). No individual taxa occurred at all 4 springs. Oligochaeta, Physidae, *Zealeuctra, Argia, Sanfilippodytes, Prionocyphon,* and *Tipula* were collected at three of the four springs. Planorbidae, *Caecidotea*, Odontoceridae, *Helicopsyche*, *Microvelia, Chauliodes, Heterosternuta sulphuria*, Sphaeridiinae, Tanypodinae, and *Hexatoma* were collected from two springs. *Lirceus*, Baetidae, Leptophlebiidae, *Ameletus, Polycentropus, Pycnopsyche, Pseudostenophylax, Sialis, Agabus, Copelatus, Optioservus, Ectopria, Ptycoptera,* Tabanidae, *Myxosaurgus,* and Limoniinae were collected from only one spring. Taxa represented by singletons (i.e. only one individual collected) included *Lirceus, Polycentropus, Pycnopsyche, Sialis*, *Copelatus*, *Ptycoptera*, and Limoniinae.

The metric percent EPT (i.e. percent Ephemeroptera, Plecoptera, and Trichoptera) ranged widely among the streams from 5.13 percent at spring 1 to 37.2 percent at spring 3. Taxon dominance in the springs is shown by the metric *percent 2 dominant*, which ranged from 44.1 percent to 67.5 percent. Spring 3 had the lowest percent tolerant organisms (55.8) and the greatest number of intolerant taxa (9). In contrast, springs 1, 2 and 4 had percent tolerant organisms > 80. Spring 3 showed the lowest HBI score (4.78, Good ranking), compared to spring 1 (6.79, Fairly Poor), spring 2 (6.70, Fairly Poor), and spring 4 (6.16, Fair) (Hilsenhoff 1987). The relatively high HBI scores at springs 1, 2, and 4 reflect moderate levels of organic pollution. Based on functional feeding group metrics, the springs were comprised primarily of predators, shredders and collector gatherers, likely attributed to low flows and heavy accumulations of leaf material. The metric *percent scrapers* was relatively high at spring 3 because of the occurrence of the mayfly genus *Ameletus.* This spring also had the greatest depths and highest flow velocities, which likely provided *Ameletus* the substrate and flows necessary for filtering organic matter from the water.

Ten individuals of the endemic predaceous diving beetle *Heterosternuta sulphuria* were collected from springs 1 and 2, while *Sanfilippodytes* sp. was represented by 46 individuals collected from springs 2, 3 and 4, with Spring 3 having the largest number of *Sanfilippodytes* sp. (28 individuals).

Table 2. Macroinvertebrates collected with associated functional group, habit group, and tolerance values.

Taxon	Order	Functional group	Tolerance values	Habit	Spring 1	Spring 2	Spring 3	Spring 4
Oligochaeta	Oligochaeta	CG	8	BU	3	2	4	
Physidae	Gastropoda	CG	8	SP	3	2	4	
Planorbidae	Gastropoda	CG	7	SP		1	1	
Caecidotea	Isopoda	CG	8	SP	3			8
Lirceus	Isopoda	CG	8	SP	1			
Baetidae	Ephemeroptera	CG	5	CG			8	
Leptophlebiidae	Ephemeroptera	CG	2	CR		3		
Ameletus	Ephemeroptera	SC	1	CG			8	
Polycentropus	Trichoptera	PR	4	CG			1	
Odontoceridae	Trichoptera	SC	0	CG		1	3	
Helicopsychidae	Trichoptera	SC	5	CR	1	1		
Pycnopsyche	Trichoptera	SH	4	CR	1			
Pseudostenophylax	Trichoptera	SH	4	CG			2	
Zealeuctra	Plecoptera	SH	0	CR		2	10	3
Microvelia	Hemiptera	PR	8	CL	1	17		
Argia	Odonata	PR	8	CR	1	4	1	
Chauliodes	Megaloptera	PR	9	SP	1	1		
Sialis	Megaloptera	PR	7	SP		1		
Agabus	Coleoptera	PR	8	GN			9	
Copelatus	Coleoptera	PR	6	GN			1	
Heterosternuta sulphuria	Coleoptera	PR	6	GN	7	3		
Sanfilippodytes	Coleoptera	PR	6	GN		1	28	17
Optioservus	Coleoptera	SC	5	CG			3	
Sphaeridiinae	Coleoptera	PR	8	GN		1		4
Prionocyphon	Coleoptera	SH	6	CL	11	1		3
Ectopria	Coleoptera	SC	4	CG	1			
Tanypodinae	Diptera	PR	9	SP	3			1
Ptycoptera	Diptera	CG	7	BU	1			
Tabanidae	Diptera	PR	7	BU		2		
Myxosaurgus	Diptera	CG	9	BU		2		
Limoniinae	Diptera	SH	6	BU		1		
Hexatoma	Diptera	PR	3	CR			2	1
Tipula	Diptera	SH	5	BU	1	1	1	

Discussion

Several historical surveys focusing on springs of the Ozark region have been conducted (Hargis 1995; Mathis 1994; Webb *et al*. 1998). Hargis (1995) compared the flora, fauna, and water quality of 65 springs within the Main, Lee Creek, and Wedington Units of the Ozark National Forest (ONF) in the Boston Mountain ecoregion. In springs at HOBBS, pH, temperature, and EC were within reported ranges of those reported by Hargis (1995), and all but one DO measurement from our study was within the reported range (0.8 to 8.5 $mg \cdot L^{-1}$).

Table 3. Metrics calculated for aquatic macroinvertebrate surveys of springs at four springs at Hobbs State Park-Conservation Area. EPT = Ephemeroptera, Plecoptera and Trichoptera, CG = collector-gatherers, CF = collector-filterers, SC = scrapers, SH = shredders, PR = predators.

METRIC	Spring 1	Spring 2	Spring 3	Spring 4
Total Number of Individuals (N)	39	47	86	37
Number of Taxa	15	19	16	7
Number of EPT Taxa (EPT Taxa)	2	4	6	1
Percent EPT (%EPT)	5.13	14.89	37.21	8.11
Percent 1 Dominant Taxon	28.21	36.17	32.56	45.95
Percent 2 Dominant Taxa	46.15	44.68	44.19	67.57
Percent Tolerant Organisms	89.74	82.98	55.81	89.19
# intolerant Taxa	4.00	5.00	9.00	2.00
Percent non-insect	25.64	10.64	10.47	21.62
per CG	28.21	21.28	19.77	21.62
per CF	0.00	0.00	0.00	0.00
per SC	5.13	4.26	16.28	0.00
per SH	33.33	10.64	15.12	16.22
per PR	33.33	63.83	48.84	62.16
Hilsenhoff Biotic Index	6.79	6.70	4.78	6.16

The DO measurement at HOBBS that fell outside that range was spring 3, where relatively higher discharge occurred and water was relatively turbulent, flowing over bedrock slides. Similar to the springs at HOBBS, Hargis (1995) reported that springs in the ONF had little surface flow and only a few springs had discharge rates that exceeded 0.03 $m^3 \cdot s^{-1}$.

It should be noted that because the springs we sampled were not randomly selected among all springs at HOBBS or across a larger area of interest, our range of inference is limited to only the four springs surveyed in this study. Moreover, other springs at HOBBS could fall within or outside the range of characteristics reported for these four spring systems. For general comparison, the biodiversity of springs at HOBBS was similar to that found in other springs in the ONF, with some differences. The non-insect taxa that we collected were among those previously reported from regional, low flow springs in the region. However, some non-insect taxa reported in historical surveys of Ozark springs that were not collected during our surveys were Nematomorpha, Amphipoda and Decapoda. Insect orders dominating the insect communities of springs in Hargis (1995) were Coleoptera (15 taxa), Trichoptera (13), and Diptera (11), and while the dominant orders in our study were similar, we observed lower taxonomic richness within these dominant orders (i.e. Coleoptera; 7 taxa, Diptera; 7, and Trichoptera 5).

Mathis (1994) surveyed the macroinvertebrate fauna of 3 springs in the Buffalo National River (BNR) in September and December 1993 and March 1994. Unlike the Hargis survey (Hargis 1995) and our current surveys of springs at HOBBS, the highest species richness among insect orders in springs in the BNR were Trichoptera (16 taxa), Ephemeroptera (7), and Coleoptera (7). Mathis (1994) reported the following taxa from the survey of BNR springs as invertebrates that typically occur in crenal (spring) habitats according to Hynes (1970): *Lepidostoma* sp., *Ironoquia punctatissima*, *Pycnopsyche rossi*, and *Hyallela azteca*. Of these, we collected only *Pycnopsyche* and Hargis (1995) collected *Lepidostoma* sp. and *Pycnopsyche* sp. from ONF. The 3 most abundant taxa collected during our surveys were *Sanfilippodytes* (48), *Microvelia* (18), and *Caecidotea* (12). In comparison, the 3 most abundant species collected at the 3 springs in the BNR were the caddisfly *Agapetus allini* (273 individuals) and the isopods *Lirceus hoppinae* (193) and *L. garmani* (152) (Mathis 1994). Differences in macroinvertebrate communities across BNR, ONF (i.e. Hargis 1995) and HOBBS could be attributed to higher discharge and flow velocities in BNR springs.

Webb *et al*. (1998) sampled 10 karst spring in southwestern Illinois, where a relatively small portion

of the Ozark Highlands extends east of the Mississipi River. In contrast to our study at HOBBS and the studies at the ONF and BNR where aquatic insects dominated the community, Webb *et al*. (1998) found the non-insect taxa oligochaetes, amphipods, isopods, and turbellarians exceeded all aquatic insects in abundances.

The predaceous diving beetles *Heterosternuta sulphuria* or *Sanfillipodytes* were collected across all four springs surveyed at HOBBS, with overlap in occurrences only in Spring 2. The former species recently has been documented to occur throughout small Ozark streams in northern Arkansas (Longing *et al.* 2013) and its distribution likely extends further into southern Missouri and eastern Oklahoma, while the latter has been found to frequently co-occur with *H. sulphuria*. The springs surveyed at HOBBS in this study produced the largest number of *Sanfilippodytes* sp. individuals collected to date, which is significant considering *Sanfilippodytes* was observed to be an undescribed species (R. Roughley, deceased, *pers. comm.*).

Our assessment of these four springs at HOBBS occurred in 2008, a year preceeding a major ice storm that removed much of the canopy that provided shade to these stream channels. It would be worthwhile to re-survey these systems to determine if the communities and especially the species of concern persisted following that disturbance, and to further compare the habitat and physico-chemical conditions across time.

Information developed from these surveys emphasizes the need for the continued protection of perennial spring systens at HOBBS. The occurrences of two diving beetles, *Heterosternuta sulphuria* and *Sanfilippodytes sp*., highlights the need for monitoring and conservation strategies for these species, while additional surveys of spring systems at HOBBS would provide a better understanding of how these habitats are influencing populations. Furthermore, these easily collected diving beetles could serve as biological targets to integrate with regional watershed management and conservation program initiatives. The bioassessment and documentation of species of concern from these four springs provides an initial framework for monitoring and further highlights HOBBS as an important conservation area for the preservation of the region's unique biodiversity.

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An Acoustic-Based Approach for Condition Monitoring of Pipes

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Running Title: An Acoustic-Based Approach for Conditioning Monitoring of Pipes

Abstract

Polyvinyl chloride (PVC) pipes are extensively used in municipal sewer systems. As the sewer piping networks are aging, PVC pipes are prone to developing cracks that can release toxic effluents into the environment. Traditionally, to monitor defects in PVC pipes, utility operators pass a close circuit TV (CCTV) camera mounted on a guided vehicle through the pipe. The video is observed by a trained operator who records condition of the pipe. This arrangement, suffers from two major limitations. One, it is expensive due to complex set up and second, if a pipe is blocked the guided vehicle cannot pass through its entire length. A more economical and robust system is needed that can reliably detect cracks in sewer pipes. Our approach is based on measuring acoustic signal attenuation in a cracked pipe and comparing it with attenuation in a pipe with no cracks. This study is work in progress and preliminary results from laboratory test setup are presented. Testing in actual sewer installations is being planned and results will be reported in future.

Introduction

Effective preventive maintenance on aging sewer system infrastructure to mitigate sewer system overflows (SSOs) is a major challenge for the utility operators in the United States (US). Timely detection of potential defects in sewer piping networks can significantly reduce the frequency and volume of unauthorized effluent discharges. It can also result in monetary savings to the operators due to reduced number of emergency responses and other unexpected costs. Better knowledge of structural health of underground assets also enables the utility operators to prioritize deployment of corrective maintenance resources. The condition assessment of sewer piping networks is performed by collecting data through observation, direct inspection, investigation, and monitoring. Analysis of collected data helps determine structural and operational condition of the sewer pipelines.

Historically, vitrified clay pipes (VCP) were used in municipal sewer piping networks. The VCP suffered from structural failures in expansive clay soils. Failures at the VCP pipe joints were also common due to root ingress resulting in cracked pipes. The VCP were replaced by PVC pipes in sewer installations. The PVC pipes offer advantages due to their low cost, corrosion resistance, light weight and comparative ease of installation. Despite these advantages, PVC pipes are also prone to failure due to multiple reasons and need periodic condition assessment to prevent SSOs and leaks.

Present industry standard for sewer monitoring is based on passing a robot mounted with a closed-circuit television (CCTV) camera through a pipe to assess its condition. The video output from the camera is observed by a human operator who records annotates his observations on the video. Condition assessment from video signals is indirect and heavily influenced by capabilities of the camera and observational skills of the operator. Another major limitation is that robot may not be able to go through full length of the pipe due to blockages, structural defects, or other obstacles. The CCTV based systems also require an off-road capable vehicle, electric generator, a camera mounted robot, cable with reel and a custom software with a control system. These systems are, therefore, expensive and crew-hour intensive.

The present study proposes an approach to detect cracks in a pipe by measuring attenuation of a propagating acoustic signal. A rigid-walled circular pipe (such as a circular PVC sewer pipe) behaves as a waveguide when excited by an acoustic signal. The existence of a crack introduces an impedance mismatch in the signal path that causes attenuation and reflections. A pipe with cracks suffers greater attenuation and reflection compared to a pipe with no cracks. Analytical modeling of acoustic signal propagation in live sewer networks is very complex and may be intractable due to

presence of laterals and other random variable phenomenon (such as varying level of water, root ingress, rodents, blockages, and pipe defects etc.). This study, as a preliminary work, focuses on using an empirical based approach to detect cracks by measuring difference in signal attenuation between a pipe with cracks and a pipe without any cracks.

Previous Work

Previous work on designing a real-time pipeline monitoring application using acoustic signal has led to the development of portable and rapidly-deployable SL-RAT™ (Sewer Line Rapid Assessment Tool) and SewerBatt™ systems (Murray *et al*. 2014a,b). A research group at the University of North Carolina at Charlotte (UNCC) collaborated with Charlotte Water to determine the feasibility of an acoustics based pipeline condition monitoring system to detect blockages in pipes and prevent SSOs. This work led to further research and development by a technology startup company that produced SL-RAT™ (Howitt 2012; Fishburne 2010). The project also provided valuable field data measured in operating sewer systems in Charlotte that was used for academic research (Khan 2013). The work was focused on developing deterministic and stochastic models of acoustic attenuation to characterize signal propagation in sewer pipes in the presence of random variable numbers and lengths of side branches (Khan 2016; Khan 2017).

The SL-RAT™ system works by transmitting an acoustic signal through the pipe from a manhole. The received signal is measured at the next manhole and processed to determine signal attenuation caused by blockages in the pipe. Based on the measured signal attenuation, a numerical score between 0 to 9 is assigned to a pipe segment (where 0 = fully blocked and 9 = clean). The score can be used by utility operators to plan future maintenance interventions. The SL-RAT™ does not detect the extent or location of cracks or other structural defects in a sewer pipes. Utility operators have to use CCTV system to investigate the pipe sections where structural defects are suspected based on SL-RAT™ numerical scores (e.g. a pipe with a score of 5).

The SewerBatt™ has been developed by a research group at the universities of Bradford and Sheffield in United Kingdom. The system is based on analyzing modes of acoustic signal propagation. The energy in the modes of reflected signal from blockages and other surface defects is measured to classify condition of the pipe (Horoshenkov *et al*. 2003; Podd *et al*. 2007; Yin *et al*. 2005). To deploy a SewerBatt™ system, an acoustic transducer is inserted into the pipe which comes in contact with the raw effluent. It requires thorough cleaning after each use for the safety of operators. The system has been tested on live sewers during a technology demonstration for United States Environmental Protection Agency (USEPA) (Murray *et al*. 2014b). Results indicate that in pipes with multiple laterals most of the weak reflected signal is lost leading to false condition assessments requiring further inspection by a CCTV system (Murray et al. 2014b). The limitations in SL-RAT™ and SewerBatt™ systems underscore the need for further study into use of acoustic signals to monitor structural health of sewer pipes.

Theoretical Formulation

The sound pressure of an acoustic signal transmitted through a pipe is normally measured with respect to a reference pressure in terms of Sound Pressure Level (SPL) in decibel [dB]. For a travelling wave at distance d from the source, the SPL is given by (Blackstock 2000):

$$[SPL]_R = [SPL]_0 - \alpha d. \qquad (1)$$

where $[SPL]_0$ is the reference SPL and α is attenuation coefficient representing signal loss in dB/m. The relationship in (1) can be used to determine the received SPL within a straight pipe with no laterals. The attenuation coefficient α is determined from the existing theoretical model given in (Khan 2016). The reference pressure is obtained from the measurement at the reference microphone.

The cracks add another a signal loss term (δ_T) in (1) and the propagation model becomes

$$[SPL]_R = [SPL]_0 - \alpha d - \delta_T. \qquad (2)$$

The model in (2) can be used to measure additional loss in an acoustic signal due to cracks and other surface defects.

Materials and Methods

A diagram of the proposed test setup to measure attenuation from a crack in a pipe is given in Fig. 1. The acoustic signal comprising 22 tones at one-third octave band frequencies between 50 Hz – 10 kHz is generated using MATLAB™ on a notebook which acts as a controller. The tonal frequencies are divided into three bands. A low frequency band comprising 7 frequencies between 50 Hz-200 Hz, a mid-frequency band

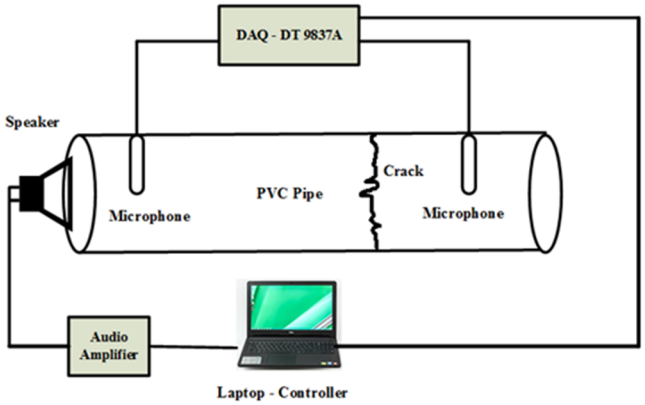

Figure 1: Laboratory Test Setup.

comprising 10 frequencies between 250 Hz- 2.0kHz; and a high-frequency band comprising 7 frequencies between 2.5 kHz to 10.0 kHz. The generated audio signals are amplified with a Samson Servo 200 Amplifier. A Tang Band (W4-1337SDF) 4" Titanium full range speaker coupled to one end of the pipe is used to transmit the audio tones through the pipe. A reference microphone (BSWA MPA 415) measures the reference signal and an output microphone placed at the other end of the pipe measures the received signal.

The difference between the signals measured at reference and output microphones gives the attenuation in the signal. A picture of the test set up is given in Fig. 2. The experimental set up includes two ten-foot sections of Schedule 40 PVC pipe (a section with a 3 mm half-diameter crack in the middle and a section without crack). The data from microphones is acquired using DT-9837A data acquisition (DAQ) module using DAQ toolbox in MATLAB™.

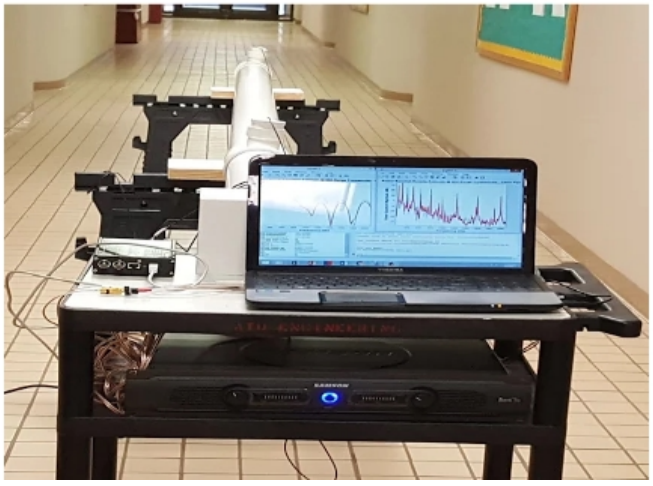

Figure 2: Test Setup.

Results

The acquired data from both microphones is analyzed to observe any changes in the pipe's frequency response due to presence of a crack. An important analysis technique to observe variation in the power present in a signal per unit frequency is the power spectral density (PSD) estimate via Welch's method given in dB/Hz. The raw PSD of the mid-frequency band signal acquired from output microphones in both clean and cracked pipes is plotted in Fig. 3. The analysis reveals that acoustic signal at 1.25 kHz is attenuated by over 3 dB in cracked pipe (from -34.7 dB to -37.8 dB). Signal loss of over 2 dB is also observed at 400 Hz. Minor losses (about 1 dB) are also observed at 500 Hz and 2.0 kHz. Minor signal gain in cracked pipe was observed at 315 Hz and 800 Hz. This is attributed to pipe resonances which occur at multiples of 35 Hz.

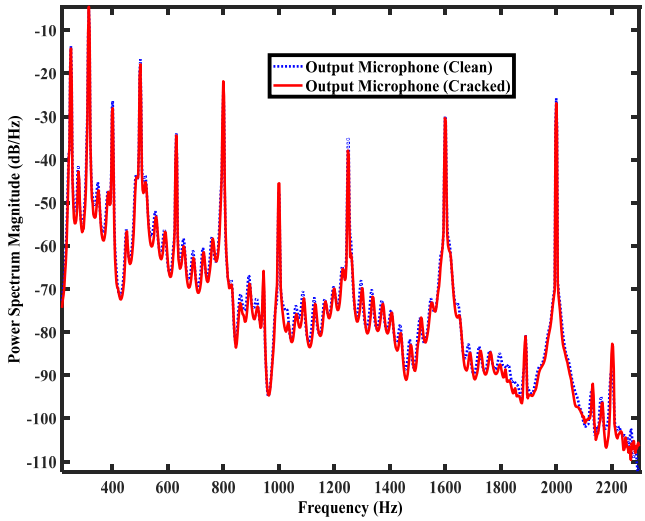

Figure 3: Raw Power Spectral Density via Welch's Method – Mid-Range Frequencies (Pipe with no cracks).

In audio signal analysis, spectrogram is an effective technique that provides visual representation of power in signal frequencies with time. Figs. 4 and 5 give spectrogram of data from output microphones in both clean and cracked pipes. Significant signal loss is observed at 1.25 kHz in cracked pipe that confirms results of PSD analysis. Pipes were also tested with audio signals other than discrete tones detailed in this paper. These included linear chirp, direct sequence spread spectrum and pink noise. The results from those tests are not presented here due to paucity of space.

It was observed during the tests that frequencies in low frequency band (especially less than 100 Hz) are not

produced efficiently by the Tang Band speaker. These frequencies, therefore, will not be used in future testing.

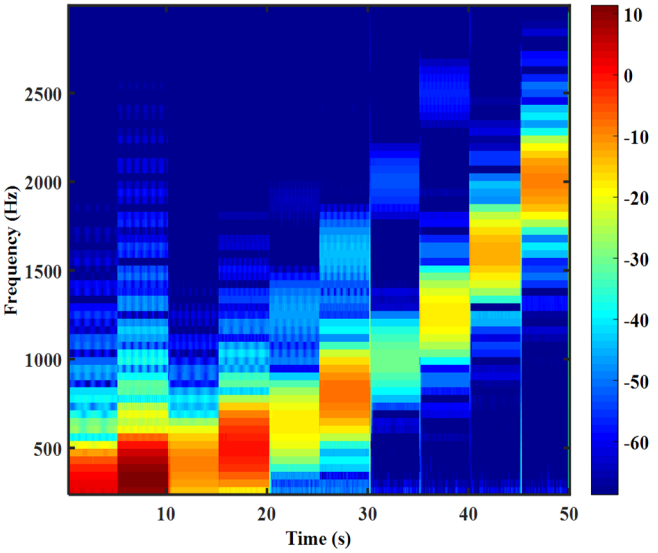

Figure 4: Spectrogram plot – Mid-Range Frequencies (Clean Pipe).

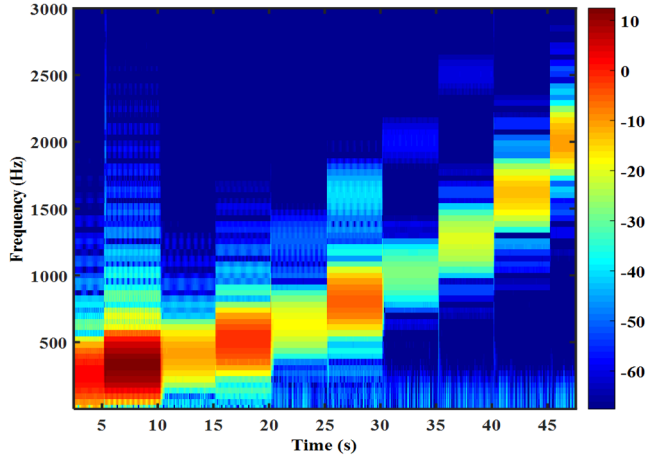

Figure 4: Raw Power Spectral Density via Welch's Method – Mid-Range Frequencies (Cracked Pipe).

Conclusion

The goal of this project was to develop an approach to monitor condition of pipes especially existence of cracks using acoustic signals. The motivation for this study has been drawn from successful use of acoustic signals to detect blockages in sewer pipes. Empirical data from extensive lab testing has enabled us to observe effect of cracks in pipes on acoustic signals at discrete frequencies. Significant signal losses were observed at 1.25 kHz and 400 Hz in a cracked pipe. Results from this study will be used to motivate and justify further testing both in laboratory and the field. The team will also use advanced techniques (such as wavelet transforms) to analyze empirical data collected during future tests. The results will enable the team to accurately predict the existence and extent of cracks and other defects in pipes.

Acknowledgements

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Sex-ratio of Miridae (Hemiptera) taken via UV light-traps in Arkansas, USA.

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Running Title: Sex-ratio of Miridae from light-traps.

Abstract

We determined the sex-ratio of 1,095 plant bugs (Hemiptera: Miridae) taken from 60 individual UV light-trap samples in Clark County, Arkansas, over a two-year period. We found that of the 21 taxa in which a sex-ratio determination could be made, 61.9% of them (13 of 21) contained a majority (over 50%) of males. Three taxa were exclusively represented by males, while two taxa were exclusively represented by females. Although taxa dependent, our data indicate that male mirids are, in general, more frequently encountered in UV light-traps. However, contrary to the notion that sparked this study (see herein) light-trap content was not represented vastly to exclusively by male individuals as the sex-ratio of the cumulative data was 62.47% males (684) and 37.53% females (411).

Introduction

A reviewer of some of our previous research sparked our investigation into this subject. The comment (paraphrased) was that we should exclude breakdown of number of male vs female plant bugs (Miridae) we found via UV light-traps in our paper as it was well known that males were mostly to exclusively collected in light-traps. This struck us as somewhat odd as while indeed the *Reuteria* species we had collected were by far majority male (86%) (Chordas *et al*. 2013), the samples from which we sorted those specimens seemed to also have an ample proportion of female mirids. However, we had no quantification of proportions at the time. We assumed that since this skewed collection of males via light-trap was well known, we could quickly find a reference. A cursory literature search using multiple key words in BIOSIS and other database research engines failed to find literature corroborating this assertion.

Our purposes herein were to quantify the mirid sex-ratio, to at least the genus level, from a large series of light-trap samples, report the ratios in the literature and evaluate the resultant ratios. We hypothesized that the overall sex-ratio of all species would be significantly skewed to the males; aligned with the comments we received previously.

Methods

During 2009 and 2010, a UV light-trapping project was conducted in the Ross Foundation Demonstration Area Forest (Clark County, Arkansas). The Ross Foundation forest was an old growth *Quercus* sp (Oak species) dominated forest with various levels of managed understory (Chordas *et al*. 2013). Although moths were the target of the study, many true bugs were captured. We use bugs from these samples for this study. The collection locality for all taxa reported herein is: **Arkansas**, *Clark County* : ~25km south west of Arkadelphia, Arkansas; forest (Ross Foundation Demonstration Area) off south side of I-30: UV light-trap [N33.937 : W-93.237], K. Benjamin collector. We sorted mirids from 60 individual UV-traps set at 19 stations within the forest between April and September of 2009 and 2010 (Table 1).

Mirids were identified to the lowest taxonomic level possible by the authors using Blatchley (1926), Henry (1976, 2015) and Knight (1941) with numbers of males and females recorded for each taxa. Taxa were grouped to at least the genus level (even if some

Table 1. UV light-traps: grouped by year, month (as a Roman numeral), date and total.

	2009		2010		
Month	Date	#UV Traps	Date	#UV traps	Total
IV			11th	1	**1**
V	27th	2	21st	4	**6**
VI	17th	10	11th & 27th	11	**21**
VII	15th	3	23rd	5	**8**
VIII	6th & 26th	16	14th	6	**22**
IX	20th	2			**2**
		= 33		= 27	**60**

Sex-ratio of Miridae from light-traps.

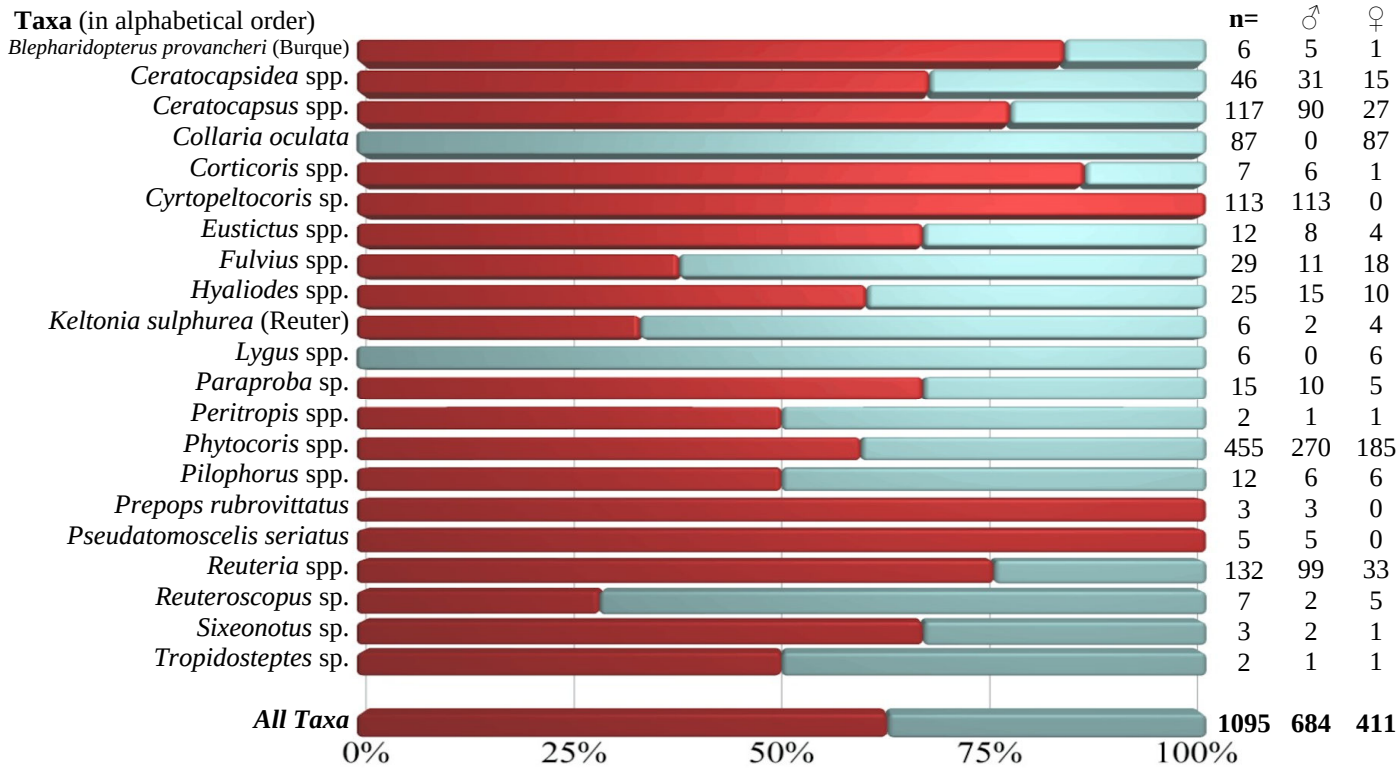

Figure 1. Stacked bar graph depicting sex-ratio for 21 taxa of mirids identified from 60 UV light-traps; dark shade = proportion (%) of males, light shade = proportion (%) of females; n= total number of individuals of that taxa identified from all 60 traps; ♂ and ♀ fraction of each total given in far right columns.

individuals of that genus group were determined to species) for male : female ratio tabulations.

Results

A total of 1,095 mirids, representing 26 genera were identified. Five taxa were represented by singletons and thus, no sex-ratio determinations were made for those taxa (they are, however, included in the "all taxa" data). Singleton taxa were: *Deraeocoris* sp. ♂, *Diphleps unica* Bergroth ♀, *Metriorrhynchromiris* sp. ♂, *Plagiognathus* sp. ♂ and *Spanagonicus albofasciatus* (Reuter) ♂. Thus, 1,090 mirids representing 21 taxa were utilized to determine sex-ratio percentages by taxa (Fig. 1).

The resultant sex-ratio for all taxa was 62.47% males and 37.53% females (Fig. 1). Of the 21 taxa, 13 (61.9%) were represented by majority males (i.e. greater than 50%), while five (23.8%) were represented by majority females and three (14.3%) had an equal 50-50 sex-ratio. The three taxa with 50-50 ratios were represented by small sample sizes; *Peritropis* sp. (n=2), *Pilophorus* spp. (n=12), *Tropidosteptes* sp. (n=2) (Fig. 1). Three taxa were represented by males only; *Cyrtopeltocoris* sp. (n=113), *Prepops rubrovittatus* (Stål) (n=3) and *Pseudatomoscelis seriatus* (Reuter) (n=5). Conversely, two taxa were represented by females only; *Collaria oculata* (Reuter) (n=87) and *Lygus* spp. (n=6) (Fig. 1).

Phytocoris was by far the dominant taxon with 455 individuals (representing 41.55% of the total number of mirids examined) with a sex ratio (male : female) of 59.34% : 40.66%. The next closest taxa in abundance, all with over 100 individuals, were *Ceratocapsus* spp. (n=117), *Cyrtopeltocoris* sp. (n=113) and *Reuteria* spp. (n=132). These four taxa accounted for approximately ¾ of the study material (74.61% of the total mirids examined) (Fig. 1) and, when combined, basically mirrored the all taxa sex-ratio with 70% : 30% (slightly male skewed being influenced by *Cyrtopeltocoris*, which were 100% males). While it was not surprising to capture only males of *Cyrtopeltocoris* sp. in light-traps, because females of the genus are brachypterous, the large number of individuals collected (n=113) was unanticipated.

Discussion

These data support the notion that overall male plant bugs are indeed more frequently attracted to and

encountered in UV light-traps over their female counterparts. However, the notion that males are exclusively or almost exclusively the representative fraction of mirids taken in UV light-traps was not supported as the male fraction of 62.47% was merely a majority. Further, that a few taxa were represented exclusively by females (*Collaria oculata*), signifies that UV light-trap sex-ratio composition in the Miridae was taxa dependent.

Given that over half of the taxa (12 out of 21 = ~57%) were represented by smaller sample sizes of 12 specimens or less, and we sorted from 60 trap samples taken over a two-year period, indicated we were less likely to take those taxa with any given single trap. Further, since the majority of these (9 of the 12) were majority male or 50:50 split, any trapping, less than the volume we examined, may very well likely, vastly or exclusively encounter the male fraction of the ultimate catch in a given light-trap. Thus, there is significantly more chance that isolated, single, or smaller trap volume will encounter a male specimen. We note that the 86% male fraction of *Reuteria* species reported by Chordas *et al*. (2013) was data from a smaller sample size than we examined for this study. Once the sample size was increased to the volume for this study, the male fraction dropped and the *Reuteria* sex-ratio herein (70% : 30%) was much closer to the all taxa ratio of 62.47% : 37.53%. We suspect that excluding the species represented by a single sex (Fig. 1), the majority of the taxa would fall close to the all taxa sex-ratio range given ample sample size.

Male mirids are frequently used for specific identifications or to confirm species identifications because they often have very distinctive parameres (claspers) and other genital components that allow for a confident identification to be made (see Knight 1941 and Henry 2015). We certainly relied on the males for species confirmations with our work on the report of five species of *Reuteria* for Arkansas (Chordas *et al.* 2013). We did not attempt to even identify any female *Reuteria* until we were comfortable with our determinations of the males using Henry's (1976) excellent key. The reliance on male specimens for identifications, especially by the authors of this paper, may tend to skew our perception that we encounter males significantly more often.

Since the data were taken from light-traps set over a six-month period with ample representation of summer and periods with favorable weather to support mirid mobility (Table 1), we did not consider temporal influences to be a factor in the resultant sex-ratio for this study. We did notice, however, that while collections of nearly all mirids spanned multiple months, a few were encountered during narrower time frames and only present during certain months (e.g. all 113 *Cyrtopeltocoris* were sorted from June samples).

Although outside the scope of this project, we noticed that a few of the genera identified in this study have no representative taxa reported in the literature for Arkansas (e.g., *Corticoris* sp., *Paraproba* sp., etc; see true bug checklist in this journal volume; Chordas (in press)). We further recognized a few species within the genera reported herein that appear to be currently unreported from Arkansas. A future plan of action will be to identify, deposit vouchers, and report any unrecorded mirid species in the literature for Arkansas.

Acknowledgments

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Geographic Distribution Records of *Macracanthorhynchus ingens* (Archiacanthocephala: Oligacanthorhynchidae) from the Raccoon, *Procyon lotor* in North America

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Running Title: *Macracanthorhynchus ingens* in North America

Macracanthorhynchus ingens is a common acanthocephalan having been reported from much of eastern North America. Although the primary definitive hosts of *M. ingens* are the raccoon, *Procyon lotor* and black bear, *Ursus americanus, M. ingens* has also been reported from ringtails (*Bassariscus astutus*), domestic dogs (*Canis familiaris*), coyotes (*Canis latrans*), hog-nosed skunks (*Conepatus leuconotus*), humans (*Homo sapiens*), eastern striped skunks (*Mephitis mephitis*), mink (*Mustela vison* and *Neovison vison*), hairy-tailed moles (*Parascalops breweri*), spotted skunks (*Spilogale putorius*), domestic swine (*Sus scrofa*), gray fox (*Urocyon cinereoargenteus*) (Richardson 2014) and more recently a bobcat (*Lynx rufus*) (Hiestand *et al*. 2014). Additionally, *M. ingens* has been reported from several reptilian and mammalian paratenic hosts (Richardson 2014). Richardson (2014) provided a faunal review of *M. ingens* showing that *M. ingens* is widely distributed throughout much of the eastern United States of America. Richardson (2014) noted however, that robust surveys of intestinal parasites of the raccoon conducted in the upper Midwestern United States (Michigan, Wisconsin, and Ohio) and in Saskatchewan Canada failed to reveal the presence of *M. ingens* (Schultz 1962, unpublished M.S. thesis, University of Michigan, East Lansing, Michigan; Hoberg and McGee 1982). Additionally, Richardson (2014) noted that *M. ingens* has not been reported from Canada or New England, north of Connecticut. In addition, there have been no vouchered reports of *M. ingens* from Missouri. Subsequent to the faunal review of Richardson (2014), specimens of *M. ingens* collected from several localities in Missouri and Ontario, Canada have been identified and are reported herein.

Specimens from Missouri raccoons were collected in the course of routine helminthological surveys. Specimens from raccoons in Ontario, Canada were taken from raccoons submitted to the Canadian Wildlife Health Cooperative, Department of Pathobiology, University of Guelph, Guelph, Canada. All specimens of *M. ingens* were collected from the small intestine and ultimately fixed in formalin or ethanol. Voucher specimens were deposited with the Division of Invertebrate Zoology, Peabody Museum of Natural History at Yale University, New Haven, Connecticut (YPM IZ).

Macaracanthorhynchus ingens was previously reported from Missouri by Monello and Grompper (2011) who reported prevalences of 2-3% based on observation of *M. ingens* eggs in fecal samples of 289 raccoons, although no worms were collected. In this study 7 of 28 (25.0%) raccoons examined from Boone County, Missouri were infected with 1 – 13 individuals of *M. ingens* with a mean intensity of 5.0. One of 27 (3.7%) raccoons examined from Cole County, Missouri was infected with 3 individuals of *M. ingens*. Two raccoons examined from Buford Pond in the Current River Conservation Area, Reynolds County, Missouri were infected with 1 and 3 individuals of *M. ingens*. Voucher specimens were deposited in the Peabody Museum of Natural History, Yale University, New Haven, Connecticut and assigned collection numbers (YPM IZ 078737-078740, 078778 and 078779). This represents the first vouchered report of *M. ingens* from Missouri.

The disparity in prevalence between raccoons in Boone and Cole Counties in central Missouri is interesting. The two counties are separated by the Missouri River with Boone county lying in the southern Alluvial Plain and Cole County lying in the northern Ozark Highlands of Missouri. Reynolds County in southern Missouri is located in the central Ozark Highlands. The finding of *M. ingens* from both southern and central Missouri, along with its occurrence in surrounding states (Richardson 2014) suggests that *M. ingens* likely occurs throughout Missouri.

Individuals of *M. ingens* were collected from raccoons collected in Cornwall, St. Thomas, Gordon Island, St Lawrence Islands National Park and Ohsweken in southern Ontario, Canada. This represents the first report of *M. ingens* from Canada. Because only representative specimens (YPM IZ 078750-078754) were provided from these raccoons submitted to the Canadian Wildlife Health Cooperative, Department of Pathobiology, University of Guelph, Guelph, Canada were provided, the prevalence and intensity of *M. ingens* in Ontario has not been determined although it appears to be uncommon.

Robust helminth surveys of raccoons by Schultz (1962, unpublished M.S. thesis, University of Michigan, East Lansing, Michigan) and Hoberg and McGee (1982) failed to reveal the presence of *M. ingens* in the upper Midwestern United States (Michigan, Wisconsin, and Ohio) and in Saskatchewan Canada. It appears that the distribution of *M. ingens* is patchy in the northern part of its range. More surveys are warranted to fully elucidate the distribution of this parasite. The availability of suitable intermediate hosts may be an important primary factor in determining the range of *M. ingens*. The primary intermediate host of *M. ingens* appears to be Spirobolid millipedes (Crites 1964; Fahnestock 1985a,b; Richardson 2006; Richardson *et al*. 2016) although beetles and woodroaches appear to also be competent intermediate hosts (Moore 1946; Elkins and Nickol 1983; Richardson 2014).

Specimens collected from a kinkajou, *Potos flavus*, from Carimagua, Meta, Colombia were determined by Richardson (2014) to be *M. ingens*. Further study of these specimens has led to the conclusion that they likely represent a previously undescribed species such that the occurrence of *M. ingens* in South America is questionable.

A map of the known geographic distribution of *M. ingens*, modified from Richardson (2014) is given in Figure 1. Given the known distribution of *M. ingens*, it is assumed that this parasite occurs in Iowa and Indiana, although there are no reports in the literature for these states. More surveys and raccoons and/or black bears are warranted throughout the upper Midwestern United States, New England, Canada, and Mexico to further elucidate the distribution of *M. ingens*.

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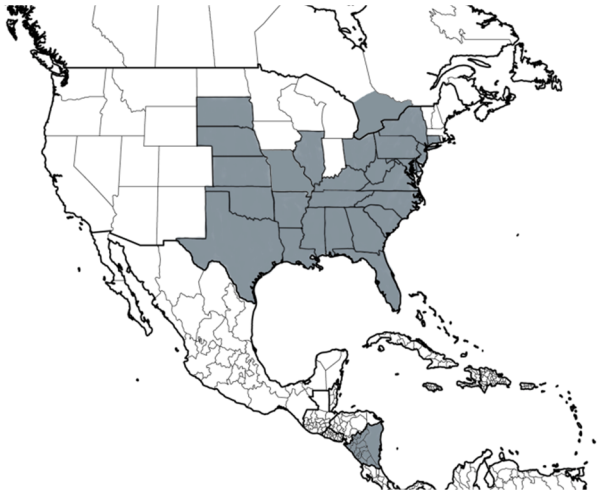

Figure 1. Documented distribution of *Macracanthorhynchus ingens* shown in gray.

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Central Nests are Heavier and Have Larger Clutches than Peripheral Nests in Cliff Swallow (*Petrochelidon pyrrohonota*) Colonies

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Running title: Within Colony Nest Site Selection and Clutch Size in Cliff Swallows

Predator avoidance is a major factor influencing nest site selection in colonial birds (Robinson 1985; Burger and Gochfield 1988; Lee and Walsh-McGee 1998). Cliff Swallows (*Petrochelidon pyrrohonota*) are common colonial nesting birds in summer in Arkansas (James and Neal 1986). They construct oblong mud nests mainly under bridges and overpasses. Old nests from previous years are frequently enhanced and reused. In some colonies, nests are in multiple horizontal tiers due to high demand for sites (Brown and Brown 1995). Colony selection in these swallows is closely related to the historical nesting success of the colony (Brown *et al*. 2000), but little is known of nest site selection within colonies.

Previous studies have documented snake predation in Cliff Swallow colonies, with nests located near the edge being more vulnerable to predation than those at the center of colonies (Brown and Brown 1987; Brown 1998; Czaplewski *et al*. 2012). Upon arrival at sites, the birds compete intensely for central nests, ostensibly because of increased risk of predation at peripheral nests (Brown and Brown 1995). In this study, we investigated if central nests are more coveted and preferred for reuse than peripheral nests in Cliff Swallow colonies. Since it is widely accepted that high-quality individuals occupy prime sites (Kokko 1999), we predicted that central nests will have higher clutch sizes than peripheral ones. If central nests are preferred for reuse, we predicted that these nests will have a higher mud mass, since old nests are augmented with new additions of mud. Accordingly, we tested two null hypotheses:

1. There is no significant difference in clutch size between central and peripheral nests within a colony, and
2. There is no significant difference in nest mass between central and peripheral regions of a colony.

During 2008, two Cliff Swallow colonies were observed near Fort Smith (Sebastian Co.), Arkansas. Both colonies were accessible by ladder and located on the undersides of small bridges over drainage canals. Nest contents were observed repeatedly throughout the nesting cycle (May-June) by using a dental mirror and flashlight as described in Brown and Brown (1996) and Leasure *et al*. (2010). In winter of 2012, one 33m-long site was used to measure mass of Cliff Swallow nests in various regions of the colony. This nest mass study was repeated in the same site in late summer of 2016 to augment sample size. Old nests were removed completely and the mass measured using a standard Triple-beam balance. The central region of the Cliff Swallow colony was designated arbitrarily as the middle 50% (16.5m) of the length of the colony, and the outer 50% of the region (25%, i.e., 8.25m, on each side) was designated as the peripheral region. Statistical analyses were performed using R (R core team 2016) and Statdisk (www.statdisk.org, Triola 2016).

Our results supported our hypotheses. Average clutch size in central nests (total 79 eggs) was 1.68 ± 1.25, 0-4 (mean ± STD, range) (n = 47 nests); average clutch size in peripheral nests (total 21 eggs) was 0.58 ± 1.13, 0-4 (mean ± STD, range) (n = 36 nests). This difference was significant (Wilcoxon Rank-Sum Test Statistic = 3.68 > Critical Value 1.95, $P < 0.05$). Therefore, clutch size was significantly higher in central compared to peripheral nests, suggesting that central nests are occupied by more robust individuals than peripheral ones. Since we examined nest content repeatedly in May-June, we are certain that the lower egg numbers in peripheral nests was not a result of predation.

In both 2012 and 2016, central nests were significantly heavier than peripheral nests (Fig. 1). In 2012, mass (g) of central nests was 342.98 ± 164.42, 95.5-541.5 (mean ± STD, range) (n = 30 nests); mass of peripheral nests was 234.42 ± 119.94, 76.2-280.9 (mean ± STD, range) (n = 10 nests). This difference was significant ($t = 1.84$, $P < 0.05$). In 2016, central nests

weighed 572 ± 179g, 259-1360 (mean ± STD, range) (n = 109 nests), and peripheral nests 511 ± 123, 246-830 (mean ± STD, range) (n = 86 nests). Again, the difference in the masses was statistically significant (t = 2.69, P = 0.003, one-tailed t-test). Nest mass was significantly higher in central compared to peripheral nests, suggesting that central nests are more reused than peripheral nests. Our nest masses data augments the previously reported information from only two nests (578 and 816 g; Emlen 1954). The low masses from 2012 may have been either due to inadequate sample size, or the fact that May 2012 was the third driest and the hottest May on record (National Oceanic and Atmospheric Administration 2017).

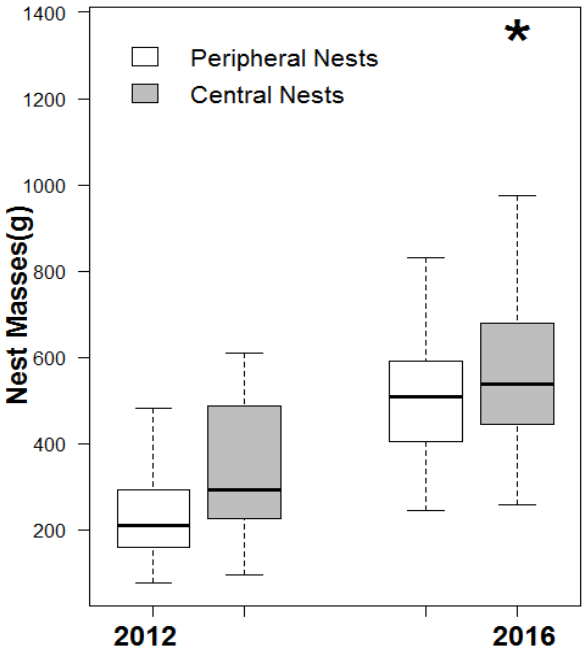

Fig. 1. Box plots comparing masses of central and peripheral nests within a colony for two seasons. The boxes represent the middle half of the data and the horizontal bars within boxes are medians. Except for the box with the outlier (*), the bottom and top whiskers represent minimum and maximum values, respectively.

Two other factors tend to suggest that central nests are preferred over peripheral nests. First, there were more central than peripheral nests in all three years of study. The proportion of central nests among all nests (0.56) was significantly greater than 0.5 in the three years combined (Test Statistic z = 2.36 > Critical z 1.64, p = 0.009; 95% Confidence Interval for the proportion is 0.52-0.63). Second, in 2016, there was just one set of 4 stacked nests at the periphery, compared to eight sets of 2-6 stacked nests in the center, indicating that birds crammed more nests (in multiple tiers) in the central zone than in the edge zones.

This study yields some insights into within-colony nest site selection and nesting success in Cliff Swallows. It suggests that there are advantages in choosing central nests over peripheral nests. Peripheral nests offer greater accessibility to predators, as is evident in Brown's (1998) description of bull snake (*Pituophis catenifer*) predation in a Nebraska colony: the snakes climb embankments on either sides of an overpass to gain access to a colony and start their predation on the extreme peripheral nests, progressively moving towards center. They eventually get satiated and stop predation, thus sparing the more interior nests. Owing to this predation pressure on peripheral nests, it is possible that the central and more coveted nesting sites are taken by more dominant and experienced individuals and/or early spring migrants (Møller 1994; Kokko 1999), forcing less experienced birds to take up more risky peripheral sites (see Petit and Petit 1996). Dominant birds may secure interior nest locations and invest more energy for nest construction, resulting in bigger and sturdier nests, than less dominant individuals. Also, since the cluttered interior nests share some walls, less energy may be required to finish a nest, and the birds can energetically afford augmenting other parts of the mud nests. This may also explain why clutch size is higher in central nests: more experienced, ostensibly robust, individuals allocate more of their energies into egg production than relatively weak and less experienced birds that may be forced to occupy the suboptimal edge nest sites.

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Sun-bathing by Greater Roadrunners – A Neglected Aspect of Their Range Extension

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Running head: Sun-bathing by Greater Roadrunners

Sun-bathing or basking is a behavior seen in birds for a variety of reasons (e.g., Kennedy 1969). One function is passive rewarming after spending the night in a state of torpor or hypothermia. While many birds can drop their body temperature during the night (McKechnie and Lovegrove 2002), passive rewarming may commonly be exhibited in only 4 species of caprimulgids and the Greater Roadrunner (*Geococcyx californianus*) (Geiser *et al*. 2004). Lowering the body temperature at night saves energy, but initially it was thought that arousing from daily torpor may be energetically costly, negating energy saved at night. However, passive rewarming expends almost no energy and is a way to rapidly raise body temperatures (Geiser *et al*. 2004). In the deserts of the Southwest, roadrunners have been reported to sun-bath, particularly after cold nights. Ohmart and Lasiewski (1971) demonstrated that this behavior significantly increased the roadrunner's body temperature following periods of hypothermia associated with those cold nights. The skin on the back of the roadrunner is black and exposed during sun-bathing by drooping wings and orienting the back towards the sun (Figure 1). This behavior is always discussed in terms of surviving cold winter nights in the desert, and has not been mentioned as a possible factor in the range extension of the Greater Roadrunner.

Beginning in the late 1930s, roadrunners began to expand their range to the east into eastern Oklahoma (Baumgartner and Baumgartner 1992), southwestern Arkansas (Baerg 1950), and northern Louisiana (Lowery 1955), possibly because of dry conditions during the Dust Bowl (Johnson 1947) and with over grazing of grasslands (Allan 1950). At least in the Ozarks, birds were associated with arid cedar glades (Brown 1963).

Using Christmas Bird Count data, Root (1988) analyzed the winter distribution of roadrunners, and concluded that it coincided with at least 140 clear (cloud-less) days, but not with temperature or precipitation. Maxon (2005) thought a combination of cloudy days, cold temperatures, prolonged snow cover, lack of woody vegetation, and scarce winter food might limit the range of roadrunners, at least to the north. While severe winters with cold temperatures and prolonged snow pack can decimate these eastern roadrunner populations (e.g., Norris and Elder 1982), the ability to survive cold nights by going into hypothermia and sun-bathing the next morning has been ignored in explanations of their range extension.

Here we document several instances of roadrunners sun-bathing after cold nights in northwestern Arkansas. Sightings of roadrunners have become more common in urban areas here within the last decade, such that roadrunners have now been seen sunbathing on several occasions.

Our first observation was made by Neal and Reynolds on the morning of 21 November 2012 in Rogers, Benton Co., Arkansas. The bird was displaying the typical sun-bathing behavior, exposing the black skin on the back to the sun (Figure 1). The bird was observed at about 8:00 after a night when the temperature was -3.3 °C. This occurred in a suburban neighborhood built around a golf course.

Figure 1. Roadrunner sunbathing on 21 November 2012 in Rogers, Arkansas. This is the classic pose with black skin on the back exposed to the sun. (Photograph by Joseph Neal).

The second observation was made by Smith and others and occurred at the Fayetteville Municipal Airport, Washington Co., on 18 December 2016 at about 7:30. The bird was sitting on a tarred road with a light dusting of snow, but moved off the road and continued to sun-bath when we stopped to look at it. The temperature the previous night was -15 °C and the temperature was only -9 °C at the time of observation.

A third observation was made by Neal and Reynolds on the side of the road near the Rocky Branch Marina on Beaver Lake, Benton Co., on 7 January 2017. The temperature at the time of the sighting was -6.1 °C. A lot of mobbing by American Crows (*Corvus brachyrhynchos*) appeared nearby. The roadrunner was sunning in an open patch of lawn adjacent the road and a thicket composed mostly of Eastern red cedar (*Juniperus virginiana*). Suddenly it stopped sunning and just froze in place, and squatted down. Then the mob got a lot louder, and as the roadrunner started to dash into a nearby cedar thicket, a Red-tailed Hawk (*Buteo jamaicensis*) swooped down on it, just missing the roadrunner as it disappeared into the thicket. Maxon (2005) was of the opinion that roadrunners were too fast for diurnal predators, but they would appear to be vulnerable to predators if sun-bathing while coming out of hypothermia. There are reports of hawks with dead roadrunners (Stevenson and Meitzen 1946), roadrunner remains in hawk pellets (Pache 1974), and attempts by raptors to capture roadrunners (Sutton 1977; Beal and Beal 1978).

All of our sightings occurred after nights when the temperatures were below freezing. The 18 December 2016 sighting followed the second coldest night of the month. While probably not directly associated with the range extensions of roadrunners to the east and north, the ability to go into daily hypothermia followed by passive warming the next morning would appear to be a significant adaptation associated with maintaining populations in these areas of range extension. Hughes (2011) also attributed this combination of physiological and behavioral adaptations to explain this once desert species now occupying new habitats as diverse as the foothills of the Rockies in Colorado to the pine forests of western Louisiana.

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New Records of Parasites (Apicomplexa, Nematoda, Acari, Anoplura) from Rodents in Arkansas

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Running Title: Parasites of Rodents in Arkansas

Compared to surrounding states, little is known about the coccidian parasites of rodents (McAllister and Kessler 2002; McAllister *et al*. 2008), and the ectoparasites of the wild mammals of Arkansas (Schiefer and Lancaster 1970; Whitaker and Wilson 1974; Whitaker *et al*. 2007; McAllister *et al*. 2013). Recently, limited work has been published on some ectoparasites of Arkansas rodents (McAllister *et al*. 2013; Tumlison *et al*. 2015). Here, we report information on a coccidian and some ectoparasites collected from rodents in the state.

Pocket gophers from Arkansas were collected as follows and examined for helminths and coccidian parasites: 10 Ozark pocket gophers (*Geomys bursarius ozarkensis*) were collected on 17-18 November 2012 from S of Melbourne at Lunenburg, Izard County; 16 Baird's pocket gophers (*Geomys breviceps*) were taken on 2 November 2012 from El Dorado, Union County; 5 *G. breviceps* were collected on 5 April 2013 from Bryant, Saline County; and 8 *G. breviceps* were taken on 15 April 2016 from Siloam Springs, Benton County. All were collected with Victor® Gopher kill traps. The gastrointestinal tract was examined for helminths and nematodes were fixed in 70% (v/v) ethanol and examined as temporary mounts in glycerol. Feces was collected from the rectum and placed in individual vials containing 2.5% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$) and examined by light microscopy following flotation in Sheather's sugar solution (specific gravity = 1.30). Negative samples were discarded and one positive sample with unsporulated oocysts was allowed one week of sporulation at room temperature (ca. 23°C) in a Petri dish containing a thin layer of 2.5% (w/v) $K_2Cr_2O_7$. Oocysts were concentrated again with Sheather's and examined using a compound microscope equipped with Nomarski interference-contrast (DIC) optics and were photographed and measured using Olympus Microsuite© software. Mean measurements are reported in micrometers (µm). A photovoucher of sporulated oocysts was accessioned into the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, NE as HWML 139189, Nematode parasites were also accessioned as HWML 99822.

One woodchuck (*Marmota monax*) from Benton County, 1 woodland vole (*Microtus pinetorum)* from Benton County, 4 white-footed mice (*Peromyscus leucopus;* 3 from Saline County and one from Marion County), 1 deermouse (*Peromyscus maniculatus*) from Benton County, 1 eastern fox squirrel (*Sciurus niger*) from Marion County, and 1 hispid cotton rat (*Sigmodon hispidus*) from Benton County were collected between February 2013 and May 2017 with Sherman live traps and Museum Special® snap traps baited with rolled oats. After being euthanized following American Society of Mammalogists guidelines (Sikes *et al*. 2011), hair and skin of rodents were examined for ectoparasites. Chiggers and other mites were cleared in lactophenol, slide-mounted in Hoyer's medium (Walters and Krantz 2009), and identified using Whitaker (1982). Sucking lice were identified in ethanol using Kim *et al*. (1986). Voucher specimens of hosts are deposited in the mammal collection at Henderson State University (HSU), Arkadelphia, AR. Ectoparasites are deposited in the Entomology Collection in the Department of Biology at Georgia Southern University, Statesboro, GA (accession nos. L3795; L3800; L3802; L3806)

The following parasites were found in or on these rodents:

Protista: Apicomplexa: Eimeriidae

***Eimeria geomydis* Skidmore**. Oocysts of a

coccidian matching the description of *E. geomydis* (Fig. 1) were found in 3 of 10 (30%) *G. b. ozarkensis*. Oocysts were subspheroidal, possessed a bilayered wall, measured (L × W) 13.0 × 11.9, and had a L/W ratio of 1.1. A micropyle, oocyst residuum, and polar granule were absent. Sporocysts were ovoidal and measured 7.9 × 4.5 with a L/W ratio of 1.8. In addition, a small Stieda body without substieda and parastieda bodies but a sporocyst residuum were present. In addition, 5 of 16 (31%) *G. breviceps* from Union County and 2 of 8 (25%) from Benton County harbored *E. geomydis*; none of the 5 *G. breviceps* from Saline County were infected. Skidmore (1929) originally described *E. geomydis* from plains pocket gopher (*Geomys bursarius*) from Nebraska. It has also been reported from *G. bursarius* from Missouri and Illinois, *G. breviceps* and Llano pocket gopher (*G. texensis*) from Texas (Upton *et al*. 1992), and northern pocket gophers (*Thomomys talpoides*) from New Mexico (Wilber *et al*. 1994). Here, we document a new distributional and host record for Arkansas, which harbors a subspecies of *G. bursarius* from which this coccidian has not been recorded.

Nematoda: Spiruroidea: Spirocercidae

Several spirurid nematodes, *Mastophorus muris* (Gemlin), were found in the stomach of 2 of 8 (25%) *G. breviceps* from Benton County (Fig. 2). Burnham (1953) reported *M. muris* from *G. bursarius* from Marshall County, Oklahoma. This nematode uses insects as intermediate hosts and is a cosmopolitan species primarily infecting wild and urban rodents, but also other less frequent hosts such as marsupials and carnivores (Rojas and Digiani 2003). We document the first report of *M. muris* in *G. breviceps* or from Arkansas.

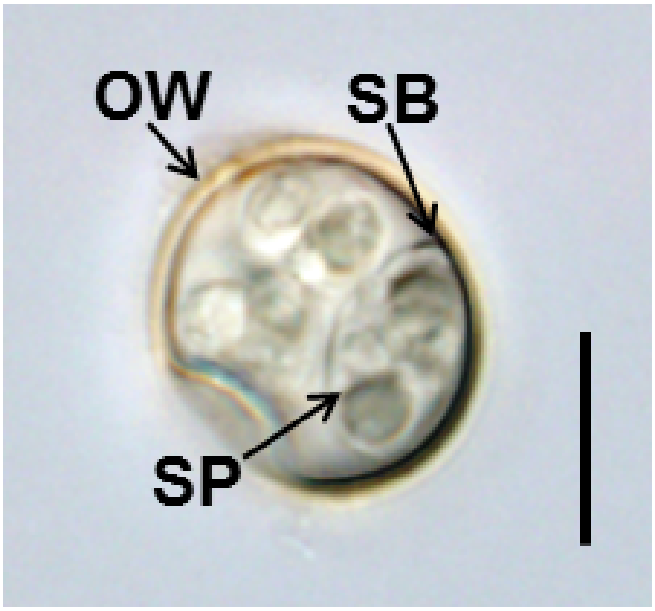

Figure 1. *Eimeria geomydis* oocyst from feces of *Geomys bursarius ozarkensis* showing oocyst wall (OW), Stieda body (SB), and sporocysts (SP). Scale bar = 5 µm.

Figure 2. Heavy infection of spirurid nematodes in the stomach of a Baird's pocket gopher (*Geomys breviceps*) from Benton County.

Acari: Trombiculidae

***Leptotrombidium peromysci* Vercammen-Grandjean and Langston**. One *L. peromysci* larva was collected from *P. leucopus* from Marion County. This chigger is associated with several species of small and medium-sized mammals in the eastern U.S. (there is also a record from South Dakota) (Walters *et al*. 2011) but this represents the first record of this species from Arkansas. Some members of the genus *Leptotrombidium* in southeast Asia and the Pacific region are vectors of *Orientia tsutsugamushi*, the causative agent of scrub typhus (chigger-borne rickettsiosis) but Nearctic members of this genus are not known to transmit any pathogens (Traub and Wisseman 1974).

***Euschoengastia peromysci* (Ewing)**. One *E. peromysci* larva was collected from *P. leucopus* from Marion County. This is a widespread and common ectoparasite of several species of small mammals across the continental U.S. (Walters *et al*. 2011). It has been reported previously from *P. leucopus* in other states (Walters *et al*. 2011), but this is the first record of this chigger from this host in Arkansas. *Euschoengastia peromysci* has previously been reported from the eastern woodrat, *Neotoma floridana* in Arkansas (Tumlison *et al*. 2015).

Laelapidae

***Androlaelaps fahrenholzi* (Berlese)**. A single male and 6 nymphs of *A. fahrenholzi* were collected from a single *M. pinetorum* from Benton County. Additionally, 9 females and 9 nymphs of *A. fahrenholzi* were collected from a single *S. hispidus* from Benton County. This is a widespread and common Nearctic ectoparasite that has been reported previously from *M. pinetorum* and *S. hispidus* in other states (Whitaker *et al*. 2007), but these are the first ectoparasite records from these hosts in Arkansas. *Androlaelaps fahrenholzi* has previously been reported from golden mice (*Ochrotomys nuttalli*) and *N. floridana* in Arkansas (Tumlison *et al*. 2015).

***Laelaps kochi* Oudemans**. One female *L. kochi* was collected from *M. pinetorum* from Benton County. Although this vole-associated ectoparasite has been collected from *M. pinetorum* in other states, this is the first time this species has been collected in Arkansas (Whitaker *et al*. 2007).

Listrophoridae

***Listrophorus pitymys* Fain and Hyland**. A male and female *L. pitymys* were collected from a single *M. pinetorum* from Benton County. This represents a new state record for this species. It has only been collected previously from hosts in New York and Rhode Island. This species has been previously reported from *M. pinetorum* (Fain and Hyland 1972, 1974).

Macronyssidae

***Ornithonyssus bacoti* (Hirst)**. The tropical rat mite was collected from a *P. maniculatus* from Benton County. Although this ectoparasite has been collected from *P. maniculatus* in other states, this is the first time it has been collected in Arkansas (Whitaker *et al*. 2007).

Ixodidae

***Ambylomma americanum* (Linnaeus)**. Six nymphs of the lone star tick were collected from *M. monax* from Benton County. This is a commonly collected tick from a variety of mammalian hosts from Arkansas (McAllister *et al*. 2016) but there are few previous records from marmots. Further west, North American marmots are often parasitized by *Ixodes marmotae* Cooley and Kohls (Durden and Keirans 1996).

***Dermacentor variabilis* (Say)**. One male American dog tick was collected from *M. monax* from Benton County. This is a commonly collected tick from a variety of mammalian hosts from Arkansas (McAllister *et al*. 2016).

Anoplura: Hoplopleuridae

***Hoplopleura hesperomydis* (Osborn)**. Five males and 13 females of *H. hesperomydis* were collected from 2 of 3 (67%) *P. leucopus* from Saline County. This sucking louse is a widespread ectoparasite in North America and Mexico on at least 9 species of *Peromyscus* and also of *Ochrotomys nuttalli* (Durden and Musser 1994) but this is the first time it has been reported from Arkansas.

In conclusion, we document a coccidian, 5 species of mites (including 2 species of chiggers), 2 species of ticks, 1 species of sucking louse, and a nematode from rodents in Arkansas. Two new host and 6 new geographic records are reported. Clearly the coccidian and ectoparasite fauna, particularly the mite fauna, of Arkansas mammals is inadequately documented. Therefore, we recommend additional surveys of the parasites from Arkansas mammals to ensure necessary documentation of vectors and reservoirs of potential zoonoses.

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Survey of Rodents within Arkansas Game and Fish Commission Wildlife Management Areas

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Running Title: Survey of Rodents within AGFC Wildlife Management Areas

Although rodents are a commonly studied group of animals, the distribution and natural history of many species within Arkansas is still not well understood or documented. Thus, we conducted this survey of rodents across Wildlife Management Areas (WMA) in Arkansas to augment current literature with new distribution records and provide notes on the natural history of rodents from Arkansas. Portions of this study (shrews) have previously been published (Pfau *et al*. 2011). Additionally, we augment recent ectoparasite records (e.g. McAllister *et al*. 2013; Tumlison *et al*. 2015) for rodents.

We collected rodents from 15 Arkansas Game and Fish Commission (AGFC) WMAs (Appendix 1). Rodents were trapped from the WMAs during three 4-night sessions from July-September in 2002 and three 3-night sessions from July-September in 2003 and 2004, using Victor© mouse traps. Five 150 m transects were set up in different habitat types on each WMA with 2 traps placed at each of 15 stations, spaced 10 m apart along the transect. All collected specimens were identified by either whole body or skulls using the keys in Sealander and Heidt (1990) and dissected to determine sex and reproductive condition. Additionally, the hair and skin of rodents were examined for ectoparasites. Ectoparasite specimens were collected and placed in vials containing 70% (v/v) ethanol. Chiggers and other mites were cleared in lactophenol, slide-mounted in Hoyer's medium (Walters and Krantz 2009), and identified using Whitaker (1982). Sucking lice were identified in ethanol using Kim *et al*. (1986). Voucher specimens of hosts are deposited in the mammal collection at Henderson State University (HSU), Arkadelphia, Arkansas. Ectoparasites are deposited in the Entomology Collection in the Department of Biology at Georgia Southern University, Statesboro, Georgia.

A total of 97 rodents was collected and identified representing 10 species (Table 1). Of note, several specimens of *Peromyscus* spp. were not identified to species due to similarities in morphological characters, requiring examination of cleaned skulls, so have been excluded from the annotated list. Seven species of rodents harbored ectoparasites. We report 2 new county records, reproductive data, and ectoparasite data below.

Microtus ochrogaster (prairie vole). — Occurs across the northern tier of counties and along the Gulf Coastal Plain with a southernmost location of Arkansas County (Sealander and Heidt 1990). A single non-reproductive female was collected from Big Lake WMA.

Microtus pinetorum (woodland vole). — Occurs throughout the state (Sealander and Heidt 1990). A single adult male was collected from Camp Robinson WMA. *Androlaelaps fahrenholzi* was collected from this individual. This mite has been previously collected from this host in other states (Whitaker *et al*. 2007).

Mus musculus (house mouse) — Occurs statewide (Sealander and Heidt 1990), usually in close association with humans. Single pregnant females were collected from both Big Lake WMA on 16 October 2002 and Holland Bottom WMA on 21 September 2004, each of which had 5 embryos.

Ochrotomys nuttalli (golden mouse). — Occurs throughout the state (Sealander and Heidt 1990). A single adult male was collected from Sulphur River WMA. This species prefers dense forested understory typical of bottomland hardwoods near riparian areas.

Oryzomys texensis (marsh rice rat). — Occurs throughout most of the state except the north central

portion (Sealander and Heidt 1990). A total of 6 marsh rice rats were collected from 3 WMAs (3 from Camp Robinson; 1 from Hurricane Lake; and 2 from Holland Bottoms). The 3 individuals from Camp Robinson represent a new county record for Faulkner County for this species (Sealander and Heidt 1990). Additionally, a single female collected on 21 Aug 2003 from Camp Robinson contained 3 embryos.

Peromyscus attwateri (Texas mouse). — Distribution is restricted to the Interior Highlands (Sealander and Heidt 1990). Four individuals (three from Gulf Mountain WMA and one from Petit Jean WMA) were collected. One of the individuals was doubly infested with a single male flea *Orchopeas leucopus* and a single female mite *Androlaelaps fahreznholzi*. Both of these ectoparasite species have been collected previously from *P. attwateri* (Tumlison *et al*. 2015). Additionally, the *P. attwateri* from Gulf Mountain WMA represent a new county record for Van Buren County (Sealander and Heidt 1990). Recently, Connior *et al*. (2013) reported this species from adjacent Searcy County.

Peromyscus gossypinus (cotton mouse). — Occurs throughout most of the state except the western half of the Springfield and Salem Plateaus (Sealander and Heidt 1990). A single adult male collected from Grandview Prairie was infested with a single female mite *Androlaelaps fahrenholzi*. This mite has been previously collected from this host in other states (Whitaker *et al*. 2007).

Peromyscus leucopus (white-footed mouse). — Occurs throughout the state (Sealander and Heidt 1990). A total of 22 individuals (4 from Camp Robison; 2 from Cedar Creek; 3 from Grandview Prairie; 3 from Harold Alexander; 1 from Henry Gray/Hurricane Lake; 1 from Madison County; and 8 from Petit Jean River). A single pregnant female collected on 24 July 2002 from Petit Jean River WMA contained 3 embryos. Additionally, 1 adult male was infested with 4 female laelapid mites *Echinonyssus utahensis*. This mite has been previously collected from this host in other states (Whitaker *et al*. 2007).

Table 1: Number and location of rodents collected from Arkansas Wildlife Management Areas

	Location														
Species	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
Microtus ochrogaster		1													
Microtus pinetorum			1												
Mus musculus		1									1				
Ochrotomys nuttalli															1
Oryzomys texensis			3							1	2				
Peromyscus attwateri								3						1	
Peromys gossypinus							1								
Peromyscus leucopus			4	2			3		3	1			1	8	
Peromyscus maniculatus			5				4	1	3					1	
Reithrodontomys fulvescens	2		2	2		1	5	1		1		1		4	2
Reithrondontomys humulis							9								
Sigmodon hispidus					5				6			1		3	

*Note: (1) Bell Slough; (2) Big Lake; (3) Camp Robinson; (4) Cedar Creek; (5) Choctaw Island; (6) Ed Gordon; (7) Grandview Prairie; (8) Gulf Mountain; (9) Harold Alexander; (10) Henry Gray Hurricane Lake; (11) Holland Bottoms; (12) Hope Upland; (13) Madison County; (14) Petit Jean; (15) Sulphur River

Peromyscus maniculatus (deer mouse). — Occurs throughout most of the state except the West Gulf Coastal Plain (Sealander and Heidt 1990). A total of 14 individuals (five from Camp Robison; four from Grandview Prairie; one from Gulf Mountain; three from Harold Alexander; and one from Petit Jean River). Two males from Camp Robinson were each infested with a single female mite *Androlaelaps fahrenholzi*. This mite has been previously collected from this host in other states (Whitaker *et al*. 2007).

Reithrodontomys fulvescens (fulvous harvest mouse). — Occurs throughout the state (Sealander and Heidt 1990). A total of 21 individuals were collected (2 from Bell Slough; 2 from Camp Robinson; 2 from Cedar Creek; 1 from Ed Gordon; 5 from Grandview Prairie; 1 from Gulf Mountain; 1 from Henry Gray/Hurricane Lake; 1 from Hope Upland; 4 from Petit Jean River; and 2 from Sulphur River). These included 3 pregnant females were collected (one from Bell Slough and two from Grandview Prairie). The female collected on 11 September 2002 from Bell Slough contained 5 embryos and the 2 from Grandview Prairie each contained 3 embryos and were collected on 7 August 2002 and 9 August 2003.

Reithrodontomys humulis (eastern harvest mouse).— Though rarely obtained during surveys, this mouse is known to occur in the upper portion of the Mississippi Alluvial Plain as far south as Lee County and the southwestern portion of the state (Sealander and Heidt 1990). A total of 9 individuals was collected from Grandview Prairie. A single pregnant female collected on 10 August 2002 contained 3 embryos and a single adult male was infested with a single female mite *Androlaelaps fahrenholzi*, which is a new host record.

Sigmodon hispidus (hispid cotton rat). — Occurs throughout the state (Sealander and Heidt 1990). A total of 15 cotton rats was collected from 4 WMAs (5 from Choctaw Island; 6 from Harold Alexander; 1 from Hope Upland; 3 from Petit Jean River). Two females were pregnant; 1 from Choctaw Island collected on 11 June 2003 had 1 embryo and 1 from Hope Upland collected on 23 July 2003 had 3 embryos. Two individuals (male from Choctaw Island and female from Harold Alexander) were each infested with a single female mite *Androlaelaps fahrenholzi*. Additionally, 1 adult male from Petit Jean River was infested by a single female sucking louse *Hoplopleura hirsuta*. These ectoparasites have been collected from this host in other states (Kim *et al*. 1986; Whitaker *et al*. 2007).

In conclusion, we record 10 species of rodents across 15 WMA's within Arkansas, with Camp Robinson, Grandview Prairie, and Petit Jean River having the highest species diversity of 5 species. Additionally, we collected 4 species of ectoparasites from rodents in Arkansas, with *R. humulis* being a new host record for *Androlaelaps fahrenholzi*. Of note, we provide the first record of reproduction for *R. humulis* within the state of Arkansas.

Acknowledgments

We thank the many AGFC wildlife biologists and technicians responsible for data collection on Wildlife Management Areas and Gary Heidt for assistance in providing training to AGFC personnel in small mammal identification.

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Appendix 1: Wildlife Management Areas (WMA) surveyed for rodents across Arkansas

WMA	County	Primary Habitat Type	Size (Hectares)
Bell Slough	Faulkner	upland pine-hardwood forests, bottomland hardwood forest, and cypress-tupelo lakes and sloughs	826
Big Lake	Mississippi	bottomland hardwoods	4,856
Camp Robinson	Faulkner	grasslands, oak savanna, upland oak/hickory forest, bottomland hardwood forests	1,630
Cedar Creek	Scott	old fields with non-native grasses and dense shrub/scrub	42
Choctaw Island	Desha	Bottomland hardwoods	3,359
Ed Gordon	Conway	herbaceous wetland waterfowl habitat, bottomland hardwoods and swamps	3,553
Grandview Prairie	Hempstead	native grasslands	1,977
Gulf Mountain	Van Buren	upland hardwoods	5,666
Harold Alexander	Sharp	oak-hickory forest interspersed with eastern red cedar glades	5,441
Henry Gray/Hurricane Lake	White	Bottomland hardwoods	6,880
Holland Bottoms	Lonoke, Pulaski	Bottomland hardwoods	2,249
Hope Upland	Hempstead	upland mixed pine and hardwood forests	856
Madison County	Madison	upland hardwoods	5,846
Petit Jean River	Yell	upland and bottomland hardwoods, pine stands, savannas and upland fields	6,305
Sulphur River	Miller	Bottomland hardwoods	7,347

Distribution of the Eastern Spotted Skunk, *Spilogale putorius*, in the Early Twentieth Century

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Running Title: Distribution of the Eastern Spotted Skunk

The eastern spotted skunk (*Spilogale putorius*) is a small carnivore that was once common across the eastern United States, but which apparently has experienced significant population declines across much of its range. Because of these declines the plains spotted skunk subspecies (*S. p. interrupta*) is being considered for federal protection as an endangered species (Gompper and Hackett 2005; U.S. Fish and Wildlife Service 2012). These declines followed expansion to the north between the Mississippi River and the Rockies in the first half of the twentieth century (Van Gelder 1959). However, early range maps were published without methodological information and combined ranges of the eastern and western spotted skunk (*Spilogale gracilis*), which were considered a single species. Thus, it is difficult to ascertain the true extent of range expansion of the eastern spotted skunk during this period (Lantz 1923; Ashbrook and Arnold 1927; Van Gelder 1959).

To document the range of the spotted skunk at the beginning of the twentieth century historic records were obtained by compiling records of spotted skunk, usually described as "civet" or "civit", captures or presence reported in Hunter, Trader, Trapper magazine from 1903-1919 and Fur News from 1907-1920; two extant magazines from this period that focused primarily on trapping. Records for which no county locality information was available were excluded from analysis. Magazine records were supplemented with museum specimens identified as eastern spotted skunks that were collected prior to 1920 that were published to VertNet (http://www.vertnet.org; accessed March 29, 2016) (Table 1).

A total of 690 magazine records and 243 museum specimens were collected (Table 1). Magazine records from Indiana (1), Ohio (1), Michigan (1), and Wisconsin (1) were excluded as outliers possibly due to misidentification or magazine editing mistakes. A range map was drawn to include all these county records. Where gaps existed between counties with records the map was drawn directly between these counties so as to include the least amount of territory without documented records as possible.

Table 1. Eastern spotted skunk magazine and museum occurrence records prior to 1920. Magazine record numbers indicate any mention of spotted skunk being present and could represent multiple individuals while museum record numbers indicate individual specimens.

State	Magazine	Museum
Alabama	13	36
Arkansas	12	0
Colorado	1	2
Florida	4	53
Georgia	15	2
Illinois	1	0
Iowa	214	8
Kansas	103	71
Kentucky	3	0
Louisiana	0	4
Minnesota	42	0
Mississippi	6	1
Missouri	77	1
Nebraska	77	8
North Carolina	0	13
Oklahoma	45	13
South Carolina	1	1
South Dakota	20	1
Tennessee	5	0
Texas	42	27
Virginia	5	1
West Virginia	2	1
Wyoming	2	0
Total	690	243

This map indicates that the spotted skunk was firmly established in southern Minnesota, southeastern South Dakota, and eastern Nebraska at this time, but with a large gap along the Mississippi River valley that is perhaps associated with the bottomland hardwood habitat found in this area (Figure 1).

Illinois has generally not been included within the range of the eastern spotted skunk although there have been reports of uncertain reliability of their presence in southern Illinois (Mohr 1943). The inclusion of this

state within their range herein is based on a letter from a trapper that reported capturing a single spotted skunk while trapping along Crooked Creek in Hancock County in the winter of 1907-1908 (Manning 1908). Unlike those records from states that were excluded, this was immediately adjacent to other parts of the range. The species appeared to have been absent from the Gulf Coastal Plain of eastern Texas, northern Louisiana, southern Arkansas, and along the Gulf Coast and most of Georgia; areas that were colonized in the subsequent forty years. The western limits of the range map should be viewed with uncertainty in areas where western spotted skunk populations may overlap. Interestingly, the map generally represents the distribution of the three subspecies of eastern spotted skunks (Van Gelder 1959).

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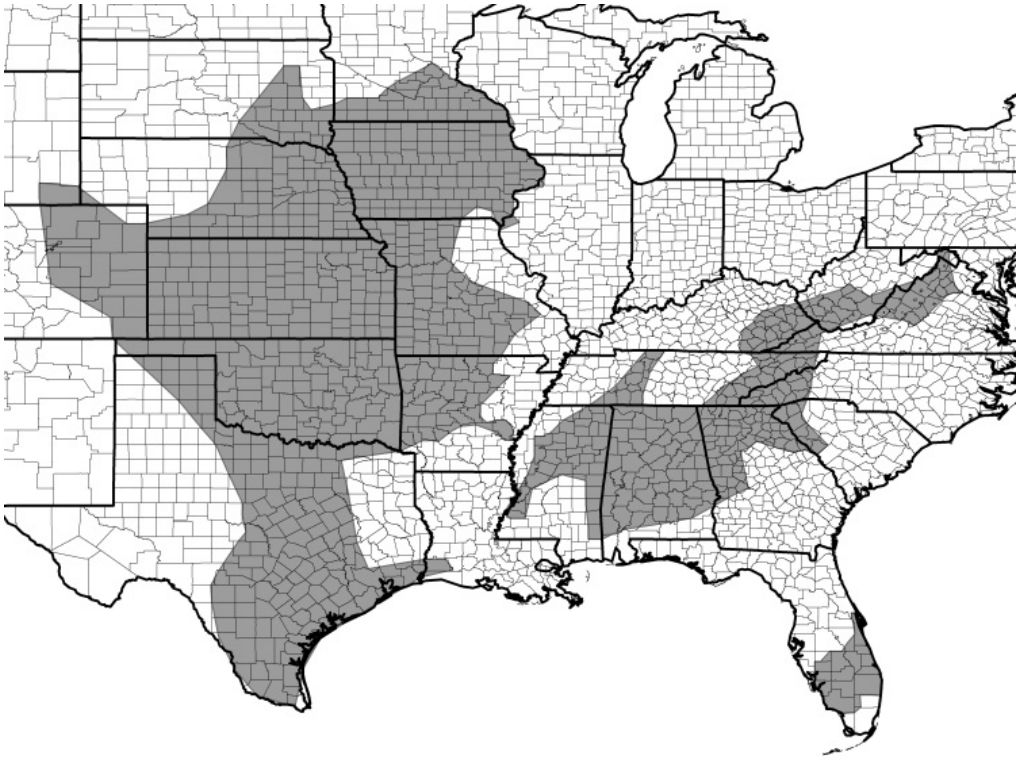

Figure 1. Distribution of the eastern spotted skunk 1900-1920.

Anatomical Distribution of *Clinostomum* Metacercariae in the Tissues of Pond-Raised Channel Catfish (*Ictaluris puctatus*)

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Running title: *Clinostomum* in Channel Catfish

Previously Daly *et al*. (2007) found that the distribution of *Clinostomum marginatum* ("yellow grub") metacercariae in the mouth and gills (orobranchial cavity) of smallmouth bass (*Micropterus dolomieu*) was highly proportional to the total body metacerariae. One could use this relationship to estimate the *Clinostomum* larval abundance in a smallmouth population by counting only the number in the mouth and gills without lethal necropsy. Lorio (1989) pointed out that yellow grub in channel catfish could cause a marketing problem for catfish farmers. A simple examination of visible anatomic sites (orobronchial areas) would be helpful for catfish growers as a tool for monitoring yellow grub in their stock. An infection of yellow grub in catfish (*Ictalurus punctatus*) in a pond in Northwest Arkansas offered the opportunity to see if such an approach would be feasible and worthwhile and to see if similar tissue distribution of proportionality existed with another fish host other than smallmouth bass.

Fifty- four catfish of similar age and size (35±3.9 cm SL; range 28-45; weight 326±169 g; range 190-1215) were taken from a pond in Washington Co. in 1995 and necropsied. The recovered yellow grubs (1712 from 54 hosts) into groupings of mouth, muscles, gills, fins and internal sites and counted. Descriptive statistics and regression analysis were done with Microsoft excel 2010.

In Fig. 1. the percentage of cysts in each of the anatomical sites are seen. The majority of the cysts, (59%), are in the orobranchial visible areas of the fish (gills + mouth). The population descriptors for yellow grub in pond-raised catfish are found in Table 1. All sites but one, muscle, have SD/Mean (Index of Dispersion) ratios of much less than one indicating a random infection process. This is unusual since most helminth infections have shown a stochastic and overdispersion of cysts in a few hosts and fewer worms in most of the other hosts. The simplest explanation for this would be that the commercial pond environment would favor random association with snail-released cercariae because there are few or no areas that the host fish can establish territorial dominance that would otherwise stratify the host-parasite relationship. The muscle SD/Mean data would indicate a different infection route for that particular anatomical site. Mean intensity, i.e. removing zero infections from the calculations, did not show much difference from total population data due to the relatively small number of zero infections. Regression analysis of mean abundance with total population versus other sites were found to be highly correlative (Table 2, Fig. 2). Importantly, total population versus the visible sites (gill-mouth) showed high correlation with r= 0.89, p =3.7E-19. This data (gill + mouth = 59% of the cysts) somewhat agreed with that of Vianna *et al*. (2005), with *C. complanatum* in *Rhamdea quellan* (a Brazilian catfish) which showed 42 % of metacercariae in the head region of the host but differed from 16 different Ouachita and Ozark smallmouth infections where less grubs were found in the head region: 14 % with a range of 5-16%, (Daly *et al*. 2014). and 19% (Taber 1972).

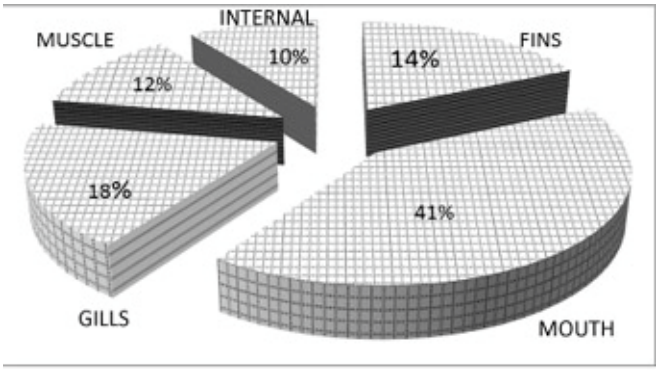

Figure 1. Percent distribution of *Clinostomum* sp. metacercarial cysts in different anatomical sites of pond-raised channel catfish (*Ictalurus punctatus*).

Table 1. Population descriptors (Bush *et al*. 1997) of *Clinostomum* sp. metacercarial cysts in different anatomical sites in channel catfish (*Ictalurus punctatus*) from a pond in Northwest Arkansas.

Mean abundance

	Total	Gill	Mouth	Mouth+Gill	Fin	Muscle	Internal
Mean	31.7	5.7	13.0	18.7	5.8	3.9	3.3
SD	21.4	5.5	8.7	11.8	5.6	5.8	3.6
Max	92	19	34	48	25	28	16
%	100	83	98	100	91	70	67
SD/Mean	0.68	0.78	0.64	0.63	0.85	1.1	0.68

Mean Intensity

	Total	Gill	Mouth	Mouth+Gill	Fin	Muscle	Internal
Mean	31.7	5.7	13.0	18.7	6.5	5.7	5.1
SD	21.4	5.3	8.6	11.8	5.5	6.2	3.4
Count	54	45	53	54	48	37	35
SD/Mean	0.68	0.78	0.5	0.63	0.85	1.1	0.67

Table 2. Regression analyses for key population descriptors of *Clinostomum* sp cysts *in Ictalurus punctatus.*

Independent Variable	Dependent Variable	X	Intercept	r	P
Total	Gill	0.16	0.7	0.60	8.3E-08
Total	Mouth	0.33	2.5	0.82	5.3E-14
Total	Gill + Mouth	1.60	1.5	0.89	3.7E-19
Total	Fins	3.20	13.1	0.83	7.6E-12
Total	Muscle	0.22	1.1	0.78	5.2E-12
Total	Internal Sites	0.09	0.6	0.50	1.0E-04

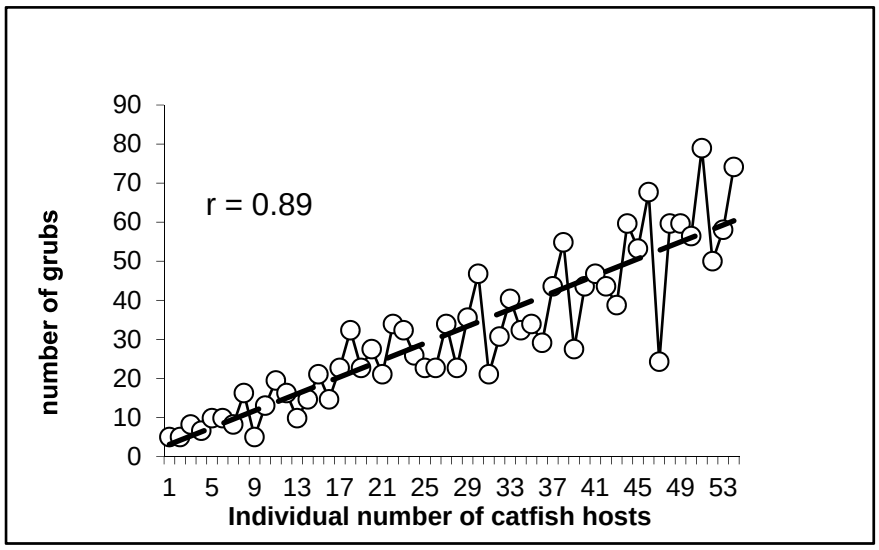

Figure 2. Regression analysis of total yellow grubs versus gill-mouth yellow grubs. Dashed line is actual total number of grubs and the circles represent the predicted total grubs calculated from regression coefficients.

In conclusion, this study shows that proportionality of *Clinostomum* larval infections exists between anatomical sites in commercial catfish as well as in smallmouth bass and also in an acanthocephalan infection of a microcrustacean. (Daly *et al*. 2014; Daly and Wagner 2016). Furthermore, McAllister *et al*. 2010 used this technique for estimating yellow grub in largemouth bass from a pond that did not require lethal necropsying of a highly valued host. Thus, counting visible grubs in the head region without necropsy gives a good estimate of the total worm burden and can be a useful tool for survey work and for catfish farmers who would not have to sacrifice economically valuable stock in order to monitor for yellow grub infections.

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Literature record checklist of true bugs (Hemiptera) for Arkansas, U.S.A., as of 2018

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Running Title: Checklist of Arkansas true bugs

The most recent catalog of the true bugs of the United States and Canada was published 30 years ago (Henry and Froeschner 1988) (Note: since this journal is printed and disseminated in April following the year of submission, the date on this paper says 2017, but was distributed in April of 2018, thus it has indeed been 30 years). The 1988 catalog continues to be a valuable resource for true bug records. In addition to species listed in the catalog, more than 150 true bug species have been added as new Arkansas records in various literature. The volume of scattered records, thus, renders it a daunting task to determine if a true bug species is or is not currently reported in the literature for Arkansas. This contribution serves to provide a single, current tabulation of Arkansas true bug literature records. This updated checklist also incorporates species previously reported from Arkansas but not included in the 1988 catalog.

Hemiptera are all listed alphabetically by family (**bold** + (# of species reported)), then by genus (indented), then by species within genus. Superscript after each species indicates a literature record for that species; in-text literature citations follow the checklist.

Hemiptera (true bugs) reported for Arkansas

Alydidae (3)
- *Alydus eurinus* (Say, 1825) [32]
- *Alydus pilosulus* Herrich-Schaeffer, 1848 [8]
- *Megalotomus quinquespinosus* (Say, 1825) [7]

Anthocoridae (4)
- *Cardiastethus assimilis* (Reuter, 1871) [8]
- *Macrotracheliella nigra* Parshley, 1917 [32]
- *Orius insidiosus* (Say, 1832) [20]
- *Xylocoris sordidus* (Reuter, 1871) [7]

Aradidae (20)
- *Acaricoris ignotus* Harris & Drake, 1944 [32]
- *Aneurus pygmaeus* Kormilev, 1966 [43]
- *Aradus acutus* Say, 1832 [43]
- *Aradus approximatus* Parshley, 1921 [40]
- *Aradus cincticornis* Bergroth, 1906 [43]
- *Aradus crenatus* Say, 1832 [43]
- *Aradus duzeei* Bergroth, 1892 [40]
- *Aradus falleni* Stål, 1860 [32]
- *Aradus ornatus* Say, 1832 [40]
- *Aradus robustus* Uhler, 1871 [43]
- *Aradus similis* Say, 1832 [40]
- *Quilnus niger* (Stål, 1873) [43]
- *Mezira emarginata* (Say, 1832) [32]
- *Mezira granulata* (Say, 1832) [43]
- *Mezira lobata* (Say, 1832) [43]
- *Mezira sayi* Kormilev, 1982 [43]
- *Neuroctenus elongatus* Osborn, 1903 [40]
- *Neuroctenus pseudonymus* Bergroth, 1898 [40]
- *Neuroctenus simplex* (Uhler, 1876) [43]
- *Notapictinus aurivilli* (Bergroth, 1887) [40]

Belostomatidae (7)
- *Belostoma flumineum* Say, 1832 [32]
- *Belostoma fusciventre* (Defour, 1863) [24]
- *Belostoma lutarium* (Stål, 1855) [32]
- *Belostoma testaceum* (Leidy, 1847) [24]
- *Lethocerus americanus* (Leidy, 1847) [24]
- *Lethocerus griseus* (Say, 1832) [32]
- *Lethocerus uhleri* (Montandon, 1896) [32]

Berytidae (4)
- *Jalysus spinosus* (Say, 1824) [32]
- *Jalysus wickhami* Van Duzee, 1906 [32]
- *Metacanthus multispinus* (Ashmead, 1887) [27]
- *Neoneides muticus* (Say, 1832) [32]

Blissidae (3)
- *Blissus leucopterus* (Say, 1832) [32]
- *Ischnodemus rufipes* Van Duzee, 1909 [46]
- *Ischnodemus slossonae* Van Duzee, 1909 [14]

Ceratocombidae (1)
- *Ceratocombus vagans* McAtee & Malloch, 1925 [2]

Cimicidae (4)
- *Cimex adjunctus* Barber, 1939 [39]
- *Cimex lectularius* Linnaeus, 1758 [32]
- *Cimex pilosellus* (Horvath, 1910) [39]
- *Cimexopsis nyctalis* List, 1925 [32]

Coreidae (19)
- *Acanthocephala declivis* (Say, 1832) [15]
- *Acanthocephala femorata* (Fabricius, 1775) [8]

Acanthocephala terminalis (Dallas, 1852) [7]
Anasa armigera (Say, 1825) [13]
Anasa tristis (De Geer, 1775) [32]
Ceraleptus americanus Stål, 1870 [32]
Chariesterus antennator (Fabricius, 1803) [7]
Chelinidea canyona Hamlin, 1923 [32]
Chelinidea vittiger Uhler, 1863 [13]
Euthochtha galeator (Fabricius, 1803) [7]
Hypselonotus punctiventris Stål, 1862 [8]
Leptoglossus clypealis Heidemann, 1910 [8]
Leptoglossus corculus (Say, 1832) [32]
Leptoglossus fulvicornis (Westwood, 1842) [7]
Leptoglossus oppositus (Say, 1832) [32]
Leptoglossus phyllopus (Linnaeus, 1767) [32]
Merocoris distinctus Dallas, 1852 [32]
Merocoris typhaeus (Fabricius, 1798) [32]
Piezogaster calcarator (Fabricius, 1803) [15]

Corixidae (15)
Corisella edulis (Champion, 1901) [6]
Corisella inscripta (Uhler, 1894) [18]
Hesperocorixa interrupta (Say, 1825) [32]
Hesperocorixa lucida (Abbott, 1916) [32]
Hesperocorixa nitida (Fieber, 1851) [23]
Hesperocorixa obliqua (Hungerford, 1925) [32]
Palmacorixa buenoi Abbott, 1913 [18]
Rhamphocorixa acuminata (Uhler, 1897) [6]
Sigara alternata (Say, 1825) [32]
Sigara hubbelli (Hungerford, 1928) [32]
Sigara modesta (Abbott, 1916) [32]
Sigara pectinata (Abbott, 1913) [25]
Trichocorixa calva (Say, 1832) [32]
Trichocorixa kanza Sailer, 1948 [32]
Trichocorixa sexcincta (Champion, 1901) [6]

Cydnidae (12)
Amnestus basidentatus Froeschner, 1960 [32]
Amnestus pallidus Zimmer, 1910 [4]
Amnestus pusillus Uhler, 1876 [32]
Amnestus spinifrons (Say, 1825) [32]
Crytomenus ciliatus (Palisot de Beauvois, 1805) [4]
Melanaethus cavicollis (Blatchley, 1924) [32]
Melanaethus pensylvanicus (Signoret, 1883) [32]
Melanaethus robustus Uhler, 1877 [4]
Melanaethus subpunctatus (Blatchley, 1926) [32]
Pangaeus bilineatus (Say, 1825) [32]
Sehirus cinctus cinctus (Palisot de Beauvois, 1811) [4]
Tominotus communis (Uhler, 1877) [4]

Cymidae (2)
Cymus angustatus Stål, 1874 [7]
Cymus luridus Stål, 1874 [32]

Gelastocoridae (1)
Gelastocoris oculatus oculatus (Fabricius, 1798) [32]

Geocoridae (3)
Geocoris pallens Stål, 1854 [32]
Geocoris punctipes (Say, 1832) [32]
Geocoris uliginosus (Say, 1832) [32]

Gerridae (15)
Aquarius nebularis Drake & Hottes, 1925 [32]
Aquarius remigis (Say, 1832) [32]
Gerris argenticollis Parshley, 1916 [32]
Gerris marginatus (Say, 1832) [32]
Limnoporus canaliculatus (Say, 1832) [32]
Metrobates alacris Drake, 1955 [32]
Metrobates hesperius Uhler, 1871 [32]
Neogerris hesione (Kirkaldy, 1902) [32]
Rheumatobates hungerfordi Wiley, 1923 [32]
Rheumatobates palosi Blatchly, 1926 [32]
Rheumatobates tenuipes Meinert, 1895 [32]
Rheumatobates trulliger Bergroth, 1915 [32]
Trepobates knighti Drake & Harris, 1928 [32]
Trepobates pictus (Herrich-Schaeffer, 1847) [32]
Trepobates subnitidus Esaki, 1926 [32]

Hebridae (4)
Hebrus burmeisteri Lethierry & Severin, 1896 [18]
Hebrus concinnus Uhler, 1894 [26]
Hebrus consolidus Uhler, 1894 [18]
Merragata brunnea Drake, 1917 [23]

Hydrometridae (2)
Hydrometra hungerfordi Torre-Bueno, 1926 [32]
Hydrometra martini Kirkaldy, 1900 [32]

Largidae (1)
Largus succinctus (Linnaeus, 1763) [15]

Lyctocoridae (1)
Lytocoris stalii (Reuter, 1871) [7]

Lygaeidae (7)
Lygaeus kalmii Stål, 1874 [15]
Kleidocerys resedae geminatus (Say, 1832) [7]
Melacoryphus facetus (Say, 1832) [8]
Neacoryphus bicrucis (Say, 1825) [15]
Neortholomus scolopax (Say, 1832) [7]
Nysius raphanus Howard, 1872 [40]
Oncopeltus fasciatus (Dallas, 1852) [8]

Mesoveliidae (1)
Mesovelia mulsanti White, 1879 [25]

Miridae (70)
Adelphocoris rapidus (Say, 1832) [32]
Agnocoris rossi Moore, 1955 [7]
Barberiella formicoides Poppius, 1914 [32]
Blepharidopterus provancheri (Burque, 1887) [32]
Bolteria luteifrons Knight, 1921 [32]
Ceratocapsidea balli (Knight, 1927) [30]
Ceratocapsidea complicata (Knight, 1927) [30]
Ceratocapsidea fusiformis Van Duzee, 1917 [30]
Ceratocapsus fuscosignatus Knight, 1927 [32]
Ceratocapsus modestus (Uhler, 1887) [17]

Ceratocapsus pumilus (Uhler, 1887) [32]
Ceratocapsus quadrispiculus Knight, 1927 [7]
Collaria oculata (Reuter, 1876) [8]
Deraeocoris aphidiphagus Knight, 1921 [32]
Deraeocoris histrio (Reuter, 1876) [7]
Deraeocoris nebulosus (Uhler, 1872) [32]
Diphleps unica Bergroth, 1924 [8]
Eustictus necopinus necopinus Knight, 1923 [8]
Fulvius slateri Wheeler, 1977 [7]
Hyaliodes harti Knight, 1941 [8]
Hyaliodes vitripennis (Say, 1832) [32]
Ilnacora stalii Reuter, 1876 [32]
Keltonia sulphurea (Reuter, 1907) [32]
Labopidea allii (Knight, 1923) [32]
Labopidea geminata (Johnston, 1930) [32]
Lopidea arkansae Knight, 1965 [32]
Lopidea confluenta (Say, 1832) [7]
Lopidea davisi Knight, 1917 [32]
Lopidea heidemanni Knight, 1917 [32]
Lopidea robiniae (Uhler, 1861) [8]
Lygus lineolaris (Palisot de Beauvois, 1818) [32]
Macrotylus amoenus Reuter, 1909 [32]
Neurocolpus jessiae Knight, 1934 [32]
Neurocolpus nubilus (Say, 1832) [32]
Parthenicus juniperi (Heidemann, 1892) [28]
Parthenicus sedumicola Henry, 2007 [28]
Parthenicus taxodii Knight, 1941 [28]
Pilophoropsidea camela (Knight, 1930) [30]
Pilophorus gracilis (Uhler, 1895) [7]
Phytocoris angustifrons Knight, 1926 [7]
Phytocoris canadensis Van Duzee, 1920 [7]
Phytocoris erectus Van Duzee, 1920 [8]
Phytocoris eximius Reuter, 1876 [7]
Phytocoris mundus Reuter, 1909 [7]
Phytocoris puella Reuter, 1876 [7]
Phytocoris quericola Knight, 1920 [7]
Plagiognathus obscurus Uhler, 1872 [7]
Plagiognathus politus Uhler, 1895 [7]
Polymerus basalis (Reuter, 1876) [32]
Prepops fraternus fraternus (Knight, 1923) [7]
Prepops insitivus (Say, 1832) [40]
Prepops rubrovittatus (Stål, 1862) [7]
Pseudatomoscelis seriatus (Reuter, 1876) [32]
Pseudoxenetus regalis (Uhler, 1890) [7]
Pycnoderes convexicollis Blatchley, 1926 [7]
Reuteria bifurcata Knight, 1939 [9]
Reuteria dobsoni Henry, 1976 [9]
Reuteria fuscicornis Knight, 1939 [9]
Reuteria querci Knight, 1939 [9]
Reuteria wheeleri Henry, 1976 [9]
Reuteroscopus ornatus (Reuter, 1876) [32]
Sixeonotus areolatus Knight, 1928 [32]
Spanagonicus albofasciatus (Reuter, 1907) [32]
Stenotus binotatus (Fabricius, 1794) [32]
Taylorilygus apicalis (Fieber, 1861) [32]
Texocoris nigrellus (Knight, 1939) [45]
Trigonotylus coelestialium (Kirkaldy, 1902) [32]
Trigonotylus tenuis Reuter, 1893 [32]
Tropidosteptes cardinalis Uhler, 1878 [8]
Tytthus wheeleri Henry, 2012 [29]

Nabidae (10)
Alloeorhynchus trimacula (Stein, 1857) [44]
Carthasis decoratus (Uhler, 1901) [44]
Hoplistoscelis confusa Kerzhner & Henry [34 (*2)]
Hoplistoscelis sericans (Reuter, 1872) [32]
Lasiomerus annulatus (Reuter, 1872) [7]
Nabis alternatus Parshley, 1922 [32]
Nabis americoferus Carayon, 1961 [7]
Nabis capsiformis Germar, 1838 [32]
Pagasa fusca (Stein, 1857) [8]
Phorticus collaris Stål, 1873 [44]

Naucoridae (1)
Pelocoris femoratus (Palisot de Beauvois, 1820) [23]

Nepidae (6)
Nepa apiculata Uhler, 1862 [22]
Ranatra australis Hungerford, 1922 [22]
Ranatra buenoi Hungerford, 1922 [22]
Ranatra fusca (Palisot de Beauvois, 1820) [22]
Ranatra kirkaldyi Torre-Bueno, 1905 [22]
Ranatra nigra Herrich-Schaeffer, 1849 [32]

Notonectidae (8)
Buenoa confusa Truxal, 1953 [12]
Buenoa margaritacea Torre-Bueno, 1908 [32]
Buenoa scimitra Bare, 1925 [32]
Notonecta indica Linnaeus, 1771 [32]
Notonecta irrorata Uhler, 1879 [32]
Notonecta raleighi Torre-Bueno, 1907 [12]
Notonecta uhleri Kirkaldy, 1897 [12]
Notonecta undulata Say, 1832 [32]

Pachygronthidae (2)
Oedancala dorsalis (Say, 1832) [7]
Phlegyas abbreviatus (Uhler, 1876) [7]

Pentatomidae (50)
Aelia americana Dallas, 1851 [32]
Alcaeorrhynchus grandis (Dallas, 1851) [32]
Amaurochrous cinctipes (Say, 1828) [32]
Apateticus cynicus (Say, 1832) [32]
Banasa dimidiata (Say, 1832) [32]
Banasa euchlora Stål, 1872 [32]
Brochymena arborea (Say, 1825) [32]
Brochymena cariosa Stål, 1872 [32]
Brochymena carolinensis (Westwood, 1837) [32]
Brochymena punctata Van Duzee, 1909 [32]
Brochymena quadripustulata (Fabricius, 1775) [32]

Chinavia hilaris (Say, 1832) [32]
Chlorochroa ligata (Say, 1832) [32]
Chlorochroa persimilis Horvath, 1908 [32]
Chlorochroa sayi (Stål, 1872) [32]
Coenus delius (Say, 1832) [32]
Coenus inermis Harris & Johnston, 1936 [32]
Cosmopepla lintneriana (Kirkaldy 1909) [32 (*3)]
Cyptocephala antiguensis (Westwood, 1837) [4 (*4)]
Dendrocoris humeralis (Uhler, 1877) [32]
Edessa bifida (Say, 1832) [32]
Euschistus ictericus (Linnaeus, 1763) [32]
Euschistus politus Uhler, 1897 [32]
Euschistus servus (Say, 1832) [32]
Euschistus tristigmus (Say, 1832) [4]
Euschistus variolarius (Palisot de Beauvois, 1817) [32]
Euthyrhynchus floridanus (Linnaeus, 1767) [32]
Halyomorpha halys (Stål, 1855) [19]
Holcostethus limbolarius (Stål, 1872) [32]
Hymenarcys aequalis (Say, 1832) [32]
Hymenarcys nervosa (Say, 1832) [32]
Mecidea major Sailer, 1952 [32]
Mecidea minor Ruckes, 1946 [32]
Menecles insertus (Say, 1832) [32]
Mormidea lugens (Fabricius, 1775) [32]
Murgantia histrionica (Hahn, 1834) [32]
Neottiglossa cavifrons Stål, 1872 [32]
Neottiglossa sulcifrons Stål, 1872 [32]
Nezara viridula (Linnaeus, 1758) [32]
Oebalus pugnax (Fabricius, 1775) [32]
Perillus bioculatus (Fabricius, 1775) [32]
Piezodorus guildinii (Westwood, 1837) [41]
Podisus maculiventris (Say, 1832) [32]
Podisus placidus Uhler, 1870 [32]
Prionosoma podopioidea Uhler, 1863 [32]
Proxys punctulatus (Palisot de Beauvois, 1818) [32]
Stiretrus anchorago (Fabricius, 1781) [32]
Thyanta calceata (Say, 1832) [32]
Thyanta custator accerra McAtee, 1919 [32]
Trichopepla semivittata (Say, 1832) [32]

Pleidae (1)
Neoplea striola (Fieber, 1844) [32]

Reduviidae (40)
Apiomerus crassipes (Fabricius, 1803) [42]
Apiomerus spissipes (Say, 1825) [32]
Arilus cristatus (Linnaeus, 1763) [7]
Barce fraterna (Say, 1832) [7]
Ctenotrachelus shermani Barber, 1930 [3]
Diaditus tejanus Giacchi, 1980 [42]
Emesaya brevipennis brevipennis (Say, 1828) [7]
Empicoris errabundus (Say, 1832) [17]
Empicoris rubromaculatus (Blackburn, 1889) [17]
Fitchia spinosula Stål, 1872 [37]
Gnathobleda litigiosa Stål, 1872 [33]
Lophoscutus prehensilis (Fabricius, 1803) [32]
Melanolestes picipes (Herrich-Schaeffer, 1846) [42]
Microtomus purcis (Drury, 1782) [7]
Narvesus carolinensis Stål, 1859 [8]
Oncerotrachelus acuminatus (Say, 1832) [8]
Oncocephalus geniculatus (Stål, 1872) [7]
Phymata americana americana Melin, 1930 [32]
Phymata pennsylvanica Handlirsch, 1897 [32]
Ploiaria carolina (Herrich-Schaeffer, 1850) [17]
Ploiaria hirticornis (Banks, 1909) [17]
Pnirontis languida Stål, 1859 [42]
Pnirontis modesta Banks, 1910 [7]
Pselliopus barberi Davis, 1912 [7]
Pselliopus cinctus (Fabricius, 1776) [42]
Pselliopus latifasciatus Barber, 1924 [42]
Pygolampus pectoralis (Say, 1832) [7]
Rasahus hamatus (Fabricius, 1781) [7]
Rhiginia cruciata (Say, 1832) [8]
Rocconota annulicornis (Stål, 1872) [7]
Saica elkinsi Blinn, 1994 [5]
Sinea diadema (Fabricius, 1776) [32]
Sinea spinipes (Herrich-Schaeffer, 1846) [32]
Sirthenea stria carinata (Fabricius, 1798) [15]
Stenopoda spinulosa Giacchi, 1969 [42]
Triatoma sanguisuga (Leconte, 1856) [32]
Zelus cervicalis Stål, 1872 [7]
Zelus luridus Stål, 1862 [32]
Zelus renardii Kolenati, 1856 [42]
Zelus tetracanthus Stål, 1862 [40]

Rhopalidae (9)
Arhyssus lateralis (Say, 1825) [32]
Arhyssus nigristernum (Signoret, 1859) [32]
Aufeius impressicollis Stål, 1870 [32]
Boisea trivittata (Say, 1825) [16]
Harmostes fraterculus (Say, 1832) [8]
Harmostes reflexulus (Say, 1832) [32]
Jadera haematoloma (Herrich-Schaeffer, 1847) [8]
Liorhyssus hyalinus (Fabricius, 1794) [32]
Niesthrea louisianica Sailer, 1961 [32]

Rhyparochromidae (24)
Antillocoris pilosulus (Stål, 1874) [32]
Atrazonotus umbrosus (Distant, 1893) [8]
Botocudo modestus (Barber, 1948) [32]
Carpilis barberi (Blatchley, 1924) [47]
Cryphula trimaculata (Distant, 1882) [14]
Cnemodus mavortius (Say, 1832) [32]
Heraeus plebejus Stål, 1874 [18]
Kolenetrus plenus (Distant, 1882) [40]
Ligyrocoris diffusus (Uhler, 1871) [32]
Malezonotus rufipes (Stål, 1874) [32]
Megalonotus sabulicola (Thomson, 1870) [10]

Myodocha serripes Oliver, 1811 [35]
Neopamera albocincta (Barber, 1953) [7]
Neopamera bilobata (Say, 1832) [7]
Ozophora picturata Uhler, 1871 [8]
Paromius longulus (Dallas, 1852) [8]
Prytanes fuscicornis (Stål, 1874) [32]
Prytanes intercisus (Barber, 1932) [32]
Pseudopachybrachius basalis (Dallas, 1852) [7]
Pseudopachybrachius vinctus (Say, 1832) [11]
Ptochiomera nodosa Say, 1832 [47]
Sisamnes claviger (Uhler, 1895) [47]
Sisamnes contractus Distant, 1893 [47]
Xestocoris nitens Van Duzee, 1906 [40]

Saldidae (4)
Micracanthia humilis (Say, 1832) [32]
Pentacora ligata (Say, 1832) [38]
Pentacora ouachita Polhemus, 1993 [38]
Saldula pallipes (Fabricius, 1794) [32]

Schizopteridae (2)
Corixidea major McAtee & Malloch, 1925 [31]
Glyptocombus saltator Heidemann, 1906 [2]

Scutelleridae (7)
Acantholomidea denticulata (Stål, 1870) [4]
Acantholomidea porosa (Germar, 1839) [21]
Diolcus chrysorrhoeus (Fabricius, 1803) [32]
Homaemus bijugis Uhler 1872 [4]
Homaemus parvulus (Germar, 1839) [32]
Stethaulax marmorata (Say, 1832) [4]
Tetyra bipunctata (Herrich-Schaeffer, 1839) [4]

Thyreocoridae (10)
Corimelaena harti Malloch, 1919 [36]
Corimelaena lateralis lateralis (Fabricius, 1803) [36]
Corimelaena marginella Dallas, 1851 [36]
Corimelaena obscura McPherson & Sailer, 1978 [36]
Corimelaena pulicaria (Germar, 1839) [32]
Galgupha aterrima Malloch, 1919 [36]
Galgupha atra Amyot & Serville, 1843 [36]
Galgupha carinata McAtee & Malloch, 1933 [36]
Galgupha loboprostethia Sailer, 1940 [32]
Galgupha ovalis Hussey, 1925 [32]

Tingidae (15)
Acalypta lillianus Torre-Bueno, 1916 [1]
Acalypta susanae Allen *et al*., 1988 [1]
Atheas mimeticus Heidemann, 1909 [32]
Corythucha aesculi Osborn & Drake, 1916 [32]
Corythucha arcuata (Say, 1832) [7]
Corythucha ciliata (Say, 1832) [7]
Corythucha cydoniae (Fitch, 1861) [32]
Corythucha marmorata (Uhler, 1878) [7]
Gargaphia solani Heidemann, 1914 [32]
Leptopharsa clitoriae (Heidemann, 1911) [32]
Leptopharsa heidemanni (Osborn & Drake, 1916) [32]
Leptopharsa oblonga (Say, 1825) [32]
Leptoypha costata Parshley, 1917 [32]
Leptoypha mutica (Say, 1832) [7]
Teleonemia nigrina Champion, 1898 [32]

Veliidae (5)
Microvelia americana (Uhler, 1884) [32]
Microvelia hinei Drake, 1920 [32]
Microvelia pulchella Westwood, 1834 [32]
Rhagovelia knighti Drake & Harris, 1927 [32]
Steinovelia stagnalis (Burmeister, 1835) [26]

This compilation totals 393 species representing 38 families of Hemiptera. I did not find literature records for 18 families (with species anticipated for Arkansas): Acanthosomatidae, Artheneidae, Dipsocoridae, Enicocephalidae, Heterogastridae, Lasiochilidae, Leptopodidae, Macroveliidae, Microphysidae, Ninidae, Ochteridae, Oxycarenidae, Piesmatidae, Plataspidae, Polyctenidae, Pyrrhocoridae, Tessaratomidae, Thaumastocoridae.

Literature used as distributional references (indicated via numerical superscript in the checklist and following) were: [1]Allen *et al*. (1988), [2]Allen & Carlton (1989), [3]Asquith (1992), [4]Barton & Lee (1981), [5]Blinn (1994), [6]Chordas *et al*. (1996), [7]Chordas *et al*. (2005), [8]Chordas *et al*. (2011), [9]Chordas *et al*. (2013), [10]Chordas *et al*. (2014), [11]Chordas *et al*. (2017), [12]Chordas & Harp (1991), [13]Chordas & Kovarik (2008)a, [14]Chordas & Kovarik (2008)b, [15]Chordas & Kremers (2009), [16]Chordas & McAllister (2015), [17]Chordas & Tumlison (2016), [18]Cochran & Harp (1990), [19]EDDMapS (2017), [20]Elkassabany *et al*. (1996), [21]Gaspar *et al*. (2015), [22]Harp (1985), [23]Harp & Harp (1980), [24]Harp & Harp (1990), [25]Harp & Hubbard (1972), [26]Harp & Robison (2006), [27]Henry (1997), [28]Henry (2007), [29]Henry (2012), [30]Henry (2015), [31]Henry *et al*. (2010), [32]Henry & Froeschner (1988), [33]Hoffman & Roble (2011), [34]Kerzhner & Henry (2008), [35]Lariviere & Larochelle (1991), [36]Lee & Barton (1983), [37]McPherson *et al*. (1992), [38] Polhemus (1993), [39]Sasse *et al*. (2016), [40]Skvarla *et al*. (2016), [41]Smith *et al*. (2009), [42]Swanson (2011), [43]Taylor & McPherson (1989), [44]Tumlison & Chordas (*in press*), [45]Wheeler (1989), [46]Wheeler (2013), [47]Wheeler (2017).

I attempted to incorporate currently valid names and nomenclatural changes [e.g. [*2]*Hoplistoscelis confusa* rather than *H. sordida*, [*3]*Cosmopepla lintneriana* instead of *C. bimaculata* (Thomas, 1865), etc]. I further attempted to exclude synonymized taxa and only included current nomenclature for each group

as best as I could determine [e.g. Lee and Barton (1983) reported *Crytomenus mirabilis*, a junior synonym of *Crytomenus ciliatus*, and Barton and Lee (1981) reported several *Thyanta* that have been synonymized (all excluded herein) and, further, a reviewer indicated the record for the Penetatomidae species [(*4)] is in need of verification; the black bug (Thyreocoridae) *Galgupha nitiduloides nitiduloides* is excluded because although listed for Arkansas in the subspecies list in [32], it is absent from the species distribution in the same reference and absent from all other thyreocorid sources I consulted, etc.,].

Literature records herein are just that, a literature record/reference, and do not necessarily represent the first literature report of that species for Arkansas. That information, however, can often be found within many of the publications cited. Efforts to document the Hemiptera fauna of Arkansas is an ongoing endeavor.

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JOURNAL
ARKANSAS ACADEMY OF SCIENCE

101st Annual Meeting April 7-8, 2017
University of Central Arkansas

Secretary's Report
MINUTES OF THE 101th MEETING

ARKANSAS ACADEMY OF SCIENCE
SPRING 2017 BUSINESS MEETING MINUTES
April 8, 2017 – 11:30 am
University of Central Arkansas, Conway

1. The meeting was called to order at 11:31am by President Edmond Wilson.

President's Report

Can you believe that the Arkansas Academy of Science has completed its 101st year? What an honor to be a president of this wonderful organization! My chief contribution to the Arkansas Academy of Science as President is that I did no harm! My job has been so easy because of the dedicated people that comprise the Executive Committee. You will hear from most of these people as they give their reports. But I just want to tell you how professional and dedicated they are beginning with this year's host, Dr. Stephen Addison who has done a marvelous job to have a such large, smooth running meeting and wonderful speaker and banquet. Also, his staff, especially Dayna Bilderback who has been so supportive and helpful keeping up with the program and records.

I can't say too much about our Treasurer, Mostafa Hemmati. The AAS Journal used to be our biggest and most draining expense. A long time ago, David Saugey began a process in improving the journal production, then Past President Collis Geren asked the University of Arkansas Library to host the Journal. What a big advancement. Collis recommended moving to an electronic production of the journal as originally suggested by David Saugey and gathered the various people who would make all this possible. But it was our Treasurer, Mostafa Hemmati who is also the Editor in Chief who worked out the details so that the AAS Journal is now our biggest money maker. He has some even more exciting news about the cost to tell you later.

(Holding up a copy of the Journal) This is a beautifully done Journal and it is a history of the flora and fauna of Arkansas and the geology and so many other things. Much credit is due to Ivan Still, the Managing Editor, for the quality of this magazine.

It is Kim Smith who keeps the historical record for our organization and our President-Elect Paneer Selvam who is in charge of the Newsletter.

Have you noticed the AAS Website? Rami Alroobi has done an excellent job improving the website and being so quick to make changes as they are needed.

And who would like to volunteer for Jess Kelly's job as coordinator for the Judging? I believe he is secure in his job function!

Abdel Bachri is so active in many of the State's science activities and is always present for helping at the Executive committee meetings.

Finally, I owe much to our Past President, Ann Willyard, who has helped to make my year run smoothly. Ann will be leaving us because of her taking an early retirement to take her husband to live close to their grandchildren. But don't count Ann out! She will continue her research from California.

Business meeting report

I would not want to fail to mention the funds that the Arkansas Academy of Science gives to support our young investigators in the State. This is something you should look into if you are interested in some funding.

Let's give a big hand of appreciation for these tireless, talented servants of the Arkansas Academy of Science!

101 year old she is! Well, we want to take a final moment to honor someone who is almost as old as the Academy itself! Dr. Doug James retired from the University of Arkansas on his 90th year last year. He has supervised the Biota contribution to the Journal for many, many years. This is such an important task and few have the knowledge in order to record all this. Let us let Doug know how much we appreciate his interest and contributions to furthering knowledge of our state and his continuing interest.

You know, Dr. James roommate in college was James Watson. Doug invited Dr. Watson to come and give a talk in the State. Dr. Watson wanted Doug to take him birding and Doug did just that. So someone asked Doug, "What do you think about your old roommate?" Doug said, "Failed Birder!"

Finally, we are very proud of the fact that AAS is able to fund these grants to students in the State to help them with their research. Jess Kelley is in charge of this project.

Our 102nd Meeting of the AAS is on April 6-7, 2018 at Arkansas State University and Dr. Andy Sustich is our host.

Let us continue our meeting.

2. Local Arrangements Committee: Steve Addison

More than 330 people registered for the meeting. 197 abstracts were received. 83 oral abstracts and 114 posters are included in the meeting book. More than half were received after the deadline for receipt. Areas of presentations included Biological Sciences, Chemistry, Physics, Engineering, Computer Science, and Geoscience.

20 universities and 10 government and other institutions were represented at the meeting.

Dayna Bilderback was an essential component for this successful meeting as well many others

3. Secretary's Report: Colis Geren

Minutes from the Executive Committee Meeting of December, 2016 minutes were reviewed and approved.

4. Treasurer's Report: Mostafa Hemmati

An accounting of the AAS for 2017 was presented and discussed by the membership. The report was reviewed by the Auditing Committee (Dr. Collis Geren and Dr. Ivan Still, who verified all calculations) (see AAS financial statement in appendix.)

5. Historian's Report: Kim Smith

This meeting at the University of Central Arkansas in Conway is the 101st annual meeting of the Arkansas Academy of Sciences. This is the 7th time that the University has hosted the Academy, the other 6 being in 1934, 1964, 1974, 1983, 1992, and 2001.

The University of Central Arkansas was founded in Conway in 1907 as the Arkansas State Normal School. As the state's only normal school at the time, UCA has historically been the primary source of teachers in Arkansas. In 1925, Arkansas State Normal School became Arkansas State Teachers College to reflect that mission. In January 1967, Arkansas State Teachers College became the State College of Arkansas. In January 1975, the State Department of Higher Education recommended State College of Arkansas be known as The University of Central Arkansas, or UCA. Today with a more academically diverse mission, UCA is noted for its nationally recognized programs in nursing, education, physical therapy, business, performing arts, and psychology and for its Honors College, one of the first in the nation.

The university comprises six colleges: the College of Fine Arts and Communication, the College of Natural Sciences and Mathematics, the College of Business, the College of Health and Behavioral Sciences, the College of Liberal Arts, and the College of Education. UCA has about 12,000 graduate and undergraduate students. The university maintains a student-to-faculty ratio of approximately 17 to 1. Over 150 undergraduate, graduate, and professional programs are offered at the university. UCA occupies over 100 buildings within its 350 acres.

In 1920 the Bears became the mascot for the teams. However, it wasn't until April 7, 1921, that the teams were called the "Bears" in print. The Bear was an appropriate symbol for the school because Arkansas' nickname was the "Bear State".

6. Journal (JAAS #70) Report: Editor-In-Chief Mostafa Hemmati

During the spring 2016 semester, 51 manuscripts

were submitted for consideration for publication in volume 70 of the Journal of the Arkansas Academy of Science (JAAS). Soon after receiving the manuscripts, all manuscripts were sent to reviewers and three Associate Editors. The reviewers sent all manuscripts and their comments back before the end of July 2016.

Reviewers' comments were sent to the authors between July 15, 2016, and July 30, 2016. That process was completed by July 30, 2016. The authors were asked to respond to the reviewers' comments and return their manuscript back to Managing Editor, Dr. Still, by August 31, 2016. That allowed more than a month of time for the authors to respond to the reviewers' comments and pay for the Journal page charges. In the same letter, the authors were asked to mail a check for their page charges as well. August 31, 2016, was also the deadline for receipt of the payment of the page charges; we had to extend the deadline up to October 15 this year.

One manuscript was rejected due to poor English, two manuscripts were rejected by reviewers, one author did not submit the final manuscript, and we did not receive the payment for page charges of one of the manuscripts. Therefore, volume 70 of the Journal will include 46 manuscripts. In the process of manuscript submission, no manuscripts were lost.

Three Associate Editors, Dr. Collis Geren, Dr. Frank Hardcastle and Dr. Rajib Choudhury, helped considerably with locating possible reviewers for the manuscripts or serving as reviewer for more than one manuscript. I am grateful for Associate Editors' assistance. All activities relating to the handling of the manuscripts were performed electronically, and on the whole this expedited the review process. Managing editor post was performed by Dr. Ivan Still and as usual he did an excellent job. The Journal was completed by December 30, 2016 and the printing of the Journal was completed by March 15, 2017. I used Russellville Printing Company.

Managing Editor Ivan Still

There were 51 manuscripts submitted for consideration of publication in volume 70 (2016) of the JAAS, obviously boosted by the publicity of the 100th meeting.

By the beginning of May these manuscripts were checked for style, grammar, format, etc, to ensure compliance with the "Instructions to Authors". One paper was rejected at this Editorial stage due extremely poor standards of English, and the authors advised to remedy those issues for resubmission in April 2017.

Abstracts were sent to potential reviewers mid to late May. Dr. Hemmati handled Physical Science papers and recruited Drs. Collis Geren, and Dr. Frank Hardcastle to serve as Associate Editors, while Biological Science manuscripts were handled by Dr. Still and Dr. Barron (Ecology/Environmental papers). All manuscripts were sent out electronically for review by the beginning of June. These were returned to the Managing Editor at the end of June/middle of July.

Authors were contacted by e-mail by the end of July 2016 at the latest and informed if their paper was accepted with the need for minor or major revision or whether their paper was rejected. Authors were asked to return their revisions to the Managing Editor electronically by August 31, with the page charges being submitted to Dr. Hemmati, Editor-in-Chief.

Two manuscripts were rejected.

One author did not submit their final manuscript, despite reminders being sent.

One was withdrawn due to lack of payment of Page Charges.

The total number of manuscripts that will published this year is 46 (a considerable improvement and hopefully the momentum of the 100th meeting will be maintained for the 101st), of which 2 were reviews, 31 were Articles, 13 were in General Note format. Volume 70 is 310 pages long (including cover pages).

I would like to thank the reviewers and Assistant/Associate Editors for their help in the preparation of volume 70: Dr. Barron was a new recruit to the Editorial Board and performed excellently.

Report on Changes in the Journal Archiving and Future Submission system.

In June 2016, Beth Juhl Librarian/Professor at UARK contacted us with regard to migrating the Arkansas Academy of Science Journal to be part of the Digital Commons-based repository that UARK was moving its institutional repository to. This platform offers some of the search engine optimization and enhanced presence in Google Scholar that we have all been discussing in the past. It also offers analytics to see how often and which articles are being accessed and downloaded (this feature is now active on the Journal webpage). Two other Academy journals (those of South Carolina and

Georgia) currently use this system, and have been used to model our electronic site.

Over the past few months Dr. Alroobi, Cedar Middleton (UARK) Cherrie Smith (bepress) and I have been working on the format of the archive including the ability to use the system to perform all the necessary steps to move article submission and e-journal publishing on-line. Cedar Middleton began the monumental task of moving the archive to the JOURNAL website: http://scholarworks.uark.edu/jaas/ at the start of November and has transferred volumes 1-8, and 45-69, with volume 70 coming on-line shortly.

This year, for submissions to Volume 71, we began accepting manuscripts electronically via the JOURNAL website: http://scholarworks.uark.edu/jaas/. For volume 71, I have also accepted manuscripts via email, direct to me. This will give all of us involved a trial run before moving to all electronic submissions in 2018.

Open Access registration

The Journal is a member of the Directory of Open Access Journals (DOAJ). Various clarifications were required by DOAJ to maintain status, including our actual open access policy, and registering a deposit policy at: http://www.sherpa.ac.uk/forms/new-journal.php. Thus, we have updated the copyright, use and licensing policy and all articles published in the JOURNAL will be made available for use the Creative Commons license: Attribution-NoDerivatives 4.0 International (CC BY-ND 4.0). The Journal was already in the SHERPA database in duplicate, and thus I am still liaising with SHERPA to work out all those issues.

These efforts all add to the visibility of the Journal across the world (as evidence by the worldwide usage reports on the Journal website).

Future issues that arise from the new archive:

I propose for ease of publishing both the electronic and hardcopy journal and maintenance of consistent page numbers for both to rearrange the layout of the journal:

The current "front pages" encompassing the meeting report will move to the back of the journal, with the manuscript Articles moving to the front, followed by the General Notes. The manuscripts will no longer be published in alphabetical order of first author, but rather in order of processing.

I propose that the "Instructions to Authors" then only be maintained on the Academy's Journal website with the link then to the Digital commons site.

These changes will streamline production editing of the Journal for future volumes. Ironically, these changes in format actually mirror some of the earlier volumes of the Journal, and mirror some of the layouts of other Academy Journals.

7. Webmaster: Rami Alroobi

Dr. Alroobi thanked the Academy for providing him this opportunity and reminded the members that this was his first year. His goal is to keep the web site simple but complete and accurate. If any members have corrections or needed additions, please contact RamiAlroobe@saumag.edu.

8. Newsletter: Panneer Selvam

Dr. Panneer Selvam provided the Newsletter Editor's Report.

The hope is still to provide two newsletters per year, but this year the spring newsletter was all that was possible. If anyone who does not currently receive the newsletter and wants to do so, contact Panneer Selvam via e-mail rps@uark.edu and you will be added to the electronic mailing list.

9. Committee Reports:

Nominations Committee: Mostafa Hemmati

Paneer Selvam inherited the presidency of the Academy, with Frank Hardcastle as President-Elect and Edmond Wilson becomes Past President. Dr. Steve Addison was the committee's nominee for Vice President. Collis Geren moved the nomination and Mostafa Hemmati seconded it. There were no nominations from the floor and Steve was unanimously approved by the membership.

Undergraduate Research Awards: Jess Kelly

Dr. Jess Kelly provided the report of the committee which received and selected this year's recipients of research awards.

Dr. Kelly reported receiving nine proposals of which three were selected consistent with the authorized budget. He noted that this year's proposals could request up to $1,000.

The recipients are:

1. Megan Cassingham, led by faculty mentor Courtney Hatch – Hendrix College. Awarded $1,000

2. Quinton Smith, led by faculty mentor Muhammed Kahn- Arkansas Tech University.

Awarded $1,000

3. Claire Turkal, led by faculty mentor Maureen McClung – Hendrix College. Awarded $850

11. Business Old and New:

The 2018 annual meeting of the Academy will be hosted by Arkansas State University. Dr. Andy Sustich will chair the meeting.

Dr. Addison reported there were 18 total Student awards approved by the judges at the 101th AAS meeting and are detailed in Appendix A.

11. Motions and Action Items:

Dr. Mostafa Hemmati requested a discretionary budget of $8,000 for the coming year for items other than for the journal. Dr. Jess Kelly moved approval and Ann Willyard provided the second. The membership moved unanimous approval.

Dr. Panneer Selvam was installed as the President for 2017-2018. He requested that anyone with suggestions for improving the Academy should e-mail him directly at rps@uark.edu. Frank Hardcastle became President-Elect, Steve Addison became Vice President, and Edmond Wilson becomes Past President.

Dr. Ann Willyard moved adjournment at 12:35pm.

Submitted by Collis Geren, Secretary on May 17, 2017.

Treasurer's Report
ARKANSAS ACADEMY OF SCIENCE
2017 FINANCIAL STATEMENT
December 14, 2017

Balance – December 14, 2017	**$142,987.74**
Balance – December 1, 2016	**$127,944.53**
Net Gain	**$15,043.21**

DISTRIBUTION OF FUNDS

Checking Account Dec. 13, 2017 Arvest Bank, Russellville	$11,512.18
PayPal Account: available funds on December 14, 2017	$703.78
PayPal Registration Account – Available Funds on Dec. 14, 2017	$54.60
Certificate of Deposit Nov. 21, 2017 Includes Phoebe and George Harp Endowment Arvest Bank, Russellville	$51,358.59
Certificate of Depoit Nov. 21, 2017 Arvest Bank, Russellville	$51,358.59
Certificate of Depoit Nov. 21, 2017 Arvest Bank, Russellville	$28,000.00
Combined interest from Arvest Bank YTD (November 21, 2017): $134.71+$134.71+0 = $269.42	
TOTAL	**$142,987.74**

INCOME

1. Transfer from Checking to CD (Oct. 13, 2017) $28,000.00

2. GIFTS RECEIVED

a. Contribution, Collis Geren	$200
	$200.00

3. INTEREST (Interest Earned Year to Date)

a. Checking Account, Arvest Bank	1290	$0
b. CD1 (Arvest Bank)	1357	$134.71
c. CD2 (Arvest Bank)	1358	$134.71
d. CD3 (Arvest Bank)	1550	$0

All interest was added to the CDs **$269.42**

4. JOURNAL

a. Page Charges	$11,500
b. One Copy of Natural Heritage	$50
c. Subscriptions, University of Arkansas	$250
	$11,800.00

5. MISCELLANEOUS INCOME

a. Refund from ATU Undergraduate Research Grant	$13.06
	$13.06

6. MEMBERSHIP

a. Associate	$15
b. Individuals	$30
c. Individual	$210
d. Institutional	$900
e. Life, Addison $500 (Check)	$500
f. Life, Kim Smith, $500 (PayPal)	-0-
	$1,655

7. MEETING INCOME

a. PayPal Transfer	$3,200
b. PayPal Transfer	$6,700
c. Registration – 8 ATU Students	$320
	$10,220

TOTAL INCOME **$23,888.06**

EXPENSES

1. STUDENT AWARDS **$1,450**

2. AWARDS (Organizations) (**December 13, 2016**)

a. Junior Science and Humanities Sym.	$400
b. Arkansas State Science Fair	$400
c. Arkansas Junior Academy of Science	$250
d. Arkansas Science Talent Search	$150
	$1,200.00

3. UNDERGRADUATE RESEARCH AWARDS

a. Dr. Hatch, Hendrix	$1,000
b. Dr. Khan , ATU	$1,000
c. Dr. McClung, Hendrix	$1,000
	$3,000.00

4. JOURNAL

a. Volume 70 Printing Cost	$3,179.53
b. $50 Refund on Extra Page Charges	$50
c. Journal Mailing Cost	$91.23
	$3,320.76

5. MISCELLANOUS EXPENSES

1. Affiliation to AAAS Dues (Jan. 30, 2017)	$150.00
2. Reimbursed Collis for Plaques	$98.78
3. Awards Mailing Cost	$18.05
4. AAS Website Cost	$119.50
5. National Association of Academies Dues (Aug.15, 2017)	$150.00
6. EXCOM Lunch and Breakfast Expenses (Mostafa)	$93.99
	$630.32

6. MEETING EXPENSES

1. Mailed $838.14 check to Dr. Adams	$838.14
	$838.14

TOTAL EXPENSES **$10,439.22**

ARKANSAS ACADEMY OF SCIENCE
COST OF JOURNAL

VOLUME	COPIES	PAGES	PRINTER CHARGE	TOT. VOL. COST	COST/ COPY	COST/ PAGE
38 (1984)	450	97	$5,562.97	$6,167.72	$13.71	$63.58
39 (1985)	450	150	$7,856.20	$8,463.51	$18.81	$56.42
40 (1986)	450	98	$6,175.20	$6,675.20	$14.23	$68.11
41 (1987)	450	116	$7,122.79	$7,811.25	$17.36	$67.34
42 (1988)	450*	116	$7,210.79	$7,710.15	$17.13	$66.47
43 (1989)	450*	119	$8,057.24	$8,557.24	$19.02	$71.91
44 (1990)	450*	136	$9,298.64	$9,798.64	$21.77	$72.05
45 (1991)	450*	136	$9,397.07	$9,929.32	$22.06	$73.01
46 (1992)	450*	116	$9,478.56	$10,000.56	$22.22	$86.21
47 (1993)	400	160	$12,161.26	$12,861.26	$32.15	$80.38
48 (1994)	450	270	$17,562.46	$18,262.46	$40.58	$67.63
49 (1995)	390	199	$14,725.40	$15,425.40	$39.55	$77.51
50 (1996)	345	158	$11,950.00	$12,640.75	$36.64	$80.00
51 (1997)	350	214	$14,308.01	$15,008.01	$42.88	$70.13
52 (1998)	350	144	$12,490.59	$13,190.59	$37.69	$91.60
53 (1999)	350	160	$13,686.39	$14,386.39	$41.10	$89.91
54 (2000)	350	160	$14,149.07	$14,849.07	$42.43	$92.81
55 (2001)	360	195	$16,677.22	$17,498.22	$48.61	$89.73
56 (2002)	350	257	$18,201.93	$19,001.93	$54.29	$73.94
57 (2003)	230	229	$14,415.12	$15,715.12	$68.33	$68.62
58 (2004)	210	144	$7,875.76	$9,175.76	$43.99	$63.72
59 (2005)	215	226	$16,239.04	$17,835.84	$82.96	$78.92
60 (2006)	220	204	$11,348.06	$12,934.30	$58.79	$63.40
61 (2007)	195	150	$8,196.84	$9,914.69	$50.84	$66.10
62 (2008)	220	166	$2,865.00	$2,967.49	$13.49	$17.88
63 (2009)	213	206	$3,144.08	$3,144.08	$14.76	$15.26
64 (2010)	232	158	$2,713.54	$2,764.30	$11.91	$17.50
65 (2011)	200	194	$2915.12	$2,963.03	$14.82	$15.27
66 (2012)	200	216	$3,087.91	$3,180.29	$15.90	$14.72
67 (2013)	200	238	$3,311.42	$3,396.32	$16.98	$14.27
68 (2014)	180	192	$2,812.75	$2,944.08	$16.36	$15.33
69 (2015)	180	170	$2,622.87	$2,622.87	$14.57	$15.43
70 (2016)	180	310	$3,179.53	$3,320.76	$18.45	$10.82

The Total Volume Cost equals the printer's charge plus the other miscellaneous charges (e.g. Mailing Costs).

- On Volume 42 the Academy received 560 copies, but the printer did not charge us for the extra 110 copies. For comparison purposes the calculated cost/copy is based on 450 copies.
- On Volume 43 the Academy received 523 copies, but the printer did not charge us for the extra 73 copies. For comparison purposes the calculated cost/copy is based on 450 copies.
- On Volume 44 the Academy received 535 copies, but the printer did not charge us for the extra 85 copies. For comparison purposes the calculated cost/copy is based on 450 copies.
- On Volume 45 the Academy received 594 copies, but the printer did not charge us for the extra 144 copies. For comparison purposes the calculated cost/copy is based on 450 copies.
- On Volume 46 the cost was greater than usual due to the high cost of a second reprinting of 54 copies by a different printer.

APPENDIX A

AWARD WINNERS FROM THE 101ST ANNIVERSARY AKANSAS ACADEMY OF SCIENCE (awardees are underlined)

UNDERGRADUATE ORAL PRESENTATION AWARDS: Biology

1st Place

Collagen Increases Tumorigenicity of Papillary Thyroid Cancer Cells Harboring BRAFV600E Mutations by Anna Sharabura; Mackenzie Gearin; Laura MacDonald. Hendrix College

UNDERGRADUATE POSTER PRESENTATION AWARDS: Biology

1st Place

Influence of Common Salt Concentrations on Detritivore Respiration by Billy Huggins; Ashton Brass; Matthew Gifford; Sally Entrekin. University of Central Arkansas

2nd Place

Evaluating the Effects of Bird Feeders on Songbird Plumage Coloration by Stetson R. Collard; Douglas G. Barron. Arkansas Tech University

GRADUATE POSTER PRESENTATION AWARDS: Biology

1st Place

A Habitat Suitability Analysis of the Red Wolf across its Historic Range by Lauren Toivonen; Matthew Gompper; Hong He. University of Missouri, Columbia

GRADUATE ORAL PRESENTATION AWARDS: Ecology

1st Place

The Use of Color as a Status Signal in the Prairie Lizard, *Sceloporus consobrinus* by Christopher Robinson; Matthew Gifford. University of Central Arkansas

2nd Place

Components of Urbanization and Urban Proximity Identify Threats to Stream Water Quality by Stephanie Stoughton; Sally Entrekin. University of Central Arkansas

2nd Place

Migration Dynamics of Ohio Shrimp, *Macrobrachium ohione*, in Arkansas by Geoffry Spooner; Reid Adams; Lindsey Lewis. University of Central Arkansas

UNDERGRADUATE ORAL PRESENTATION AWARDS: Ecology

1st Place

A Fracking Racket: Do Birds Change the Way They Sing When Experiencing Chronic Noise from Shale Gas Extraction? by Claire Turkal; Anna Claire Atkins; Charlotte Marchioni; Evan Mitchell; Maureen McClung. Hendrix College

2nd Place

Seed Dispersal in Osage Orange (Maclura pomifera) By Squirrels (Sciurus spp.) by Sophie Katz; Jessica Bonumwezi; Matthew D. Moran; Jennifer L. Penner. Hendrix College

UNDERGRADUATE ORAL PRESENTATION AWARDS: Chemistry

1st Place

Photocatalytic Sterilization of Aqueous Solutions by David Williams; Justin Barrett. Arkansas Tech University

UNDERGRADUATE POSTER PRESENTATION AWARDS: Chemistry

1st Place

Identifying the Structure of Fat-Mobilizing Substance (FMS-1) Associated with Cogenital Lipodystrophy by Mallory Bryant. Harding University

UNDERGRADUATE ORAL PRESENTATION AWARDS: Physics

1st Place

Suppression of Radiation-Induced Chromosome Damage by GT3 and the Role of Microgravity by Calla Bassett; Abdel Bachri; Rupak Pathak. Southern Arkansas University

UNDERGRADUATE POSTER PRESENTATION AWARDS: Physics

1st Place

Potential Differences in Problem Solving Approaches When Using Different Textbooks by Charles Bertram; Andrew Mason. University of Central Arkansas

2nd Place

The Langrangian and Hamiltonian formulation of the Linear Oscillator Chain by Garrott Granholm. University of Central Arkansas

1st Place

Low-Delay Rate Control for H.265/Hevc Video Compression by Reese Childer; James Palmer; Joseph Hilton; Yu Sun. University of Central Arkansas

GRADUATE POSTER PRESENTATION AWARDS: Computing Science

1st Place

A Gentleness Simulator for Surgical Dexterity Evaluation of Surgeons with Haptic Interfaces by Recep Erol; Doga Demirel; Alex Yu; Tansel Halic; Sinan Kockara, Kevin Sexton. University of Central Arkansas

UNDERGRADUATE ORAL PRESENTATION AWARDS: Geoscience

1st Place

Lithologic Character, Sequence and Diagenetic History of Lower Mississippian Tripolitiic Chert, Northern Arkansas and Southern Missouri by Sydney McKim; Jonathan Chick; Julie Cains; Forrest McFarlin; Adriana Potra. University of Arkansas, Fayetteville

APPENDIX B
RESOLUTIONS

Arkansas Academy of Science
101st Annual Meeting, 2017 Resolutions

Be it resolved that we, the membership of the Arkansas Academy of Science (AAS) offer our sincere appreciation to the University of Central Arkansas for hosting the 101st annual meeting of the Academy. We thank the local arrangements committee: Stephen Addison (Chair), Ginny Adams (Co-Chair), Ben Rowley, Robert Mauldin, Steve O'Connell, George Bratton, Ashley Hicks, Yu Sun, Dayna Bilderback, Tracy McGarrity and the Provost and Deans who supported the awards and volunteering Faculties listed in the AAS proceedings.

We sincerely thank the University of Central Arkansas for providing its facilities and service during the meeting and Aramark for the catering service.

We especially thank our keynote speaker, Dr. Sally Entrekin, for her informative talk.

The Academy recognizes the important role of our session chairs: Arijit Mukherjee (UCA), Amber Harrington (ATU), Mostafa Hemmati (ATU), Antoinette Odendaal (SAU), Jess Kelly (OBU), Carl Frederickson (UCA), Douglas Barron (ATU), Ann Willyard (HC), Jeff Allender (UCA), Douglas James (UAF), Muhammad Khan (ATU), and Tsunemi Yamashita (ATU).

Even greater appreciation and sincere gratitude is extended to our dedicated judges for the student presentations including Arijit Mukherjee (UCA), Amber Harrington (ATU), Mostafa Hemmati (ATU), Antoinette Odendaal (SAU), Jeff Kelly (OBU), Carl Frederickson (UCA), Douglas Barron (ATU), Ann Willyard (HC), Jeff Allender (UCA), Puskar Chapagain (SAU), Kari Naylor (UCA), David Sasse (AGF), Rick Noyse (UCA), Jessica Young (ATU), Dennis Province (HU), William Slaton (UCA), Jennifer Dearolf (HC), Muhammad Khan (ATU), Mikolaj Sulkowski (SAU), Mary Stewart (UAM), Stephen Addison (UCA), Martin Campbell (HSU), Amirta Puri (UCA), Matthew Young (ATU), Tammy Haselkorn (UCA), Nilu Runge (UCA), Brian Wagner (AGFC), Mariusz Gajewski (ATU), Bernard Chen (UCA), Jamie Dalton (ATU), David Dussourd (UCA), and Rahul Mehta (UCA).

We congratulate our student researchers, scientists and engineers who presented papers and posters whose efforts contribute directly to the future success of the Academy and the improvement of advancement of science in Arkansas.

The Academy recognizes its leadership and offers its thanks to this year's set of executive officers including Ed Wilson (President), Panneer Selvam (President Elect), Ann Willyard (Past President), Franklin Hardcastle (Vice President), Mostafa Hemmati (Treasurer and Journal Editor-in-Chief), Ivan Still (Journal Managing Editor), Panneer Selvam (Newsletter Editor), Rami Alroobi (Webmaster), Kimberly Smith (Historian), and Collis Geren (Secretary).

Respectfully submitted on this 8th day of April, 2017. Resolutions Committee: Ed Wilson (President), Franklin Hardcastle (Vice President), and Mostafa Hemmati (Treasurer).

2017 MEMBERSHIP

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Phoebe	Harp	Arkansas State University
Gary	Heidt	University of Arkansas-Little Rock
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Art	Johnson	Hendrix College
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Scott	Kirkconnell	Arkansas Tech University
Roger	Koeppe	University of Arkansas
Christopher	Liner	University of Arkansas
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David	Saugey	U.S. Forest Service
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Ivan	Still	Arkansas Tech University
Suresh	Thallapuranam	University of Arkansas-Fayetteville
Stanley	Trauth	Arkansas State University
Gary	Tucker	FTN Associates
Renn	Tumlison	Henderson State University
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Chris	McAllister	Eastern Oklahoma State College-Idabel
Brittany	McCall	Arkansas State University
Maureen	McClung	Hendrix University
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Rahul	Mehta	University of Central Arkansas
Jim	Rippy	University of Florida
Freddys	Rodriguez	Arkansas Tech University
Virginie	Rolland	Arkansas State University-Jonesboro
Terri	Ross	
Blake	Sasse	Arkansas Game and Fish
Salena	Sasser	Southern Illinois University
Hamed	Shojaei	Arkansas Tech University
William	Slaton	University of Central Arkansas
Kimberly	Smith	University of Arkansas- Fayetteville
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Jan	Keith	Arkansas Tech University
Susanne	Wache	South Arkansas Community College
Edmond	Wilson	Harding University

KEYNOTE ADDRESS

Arkansas streams will play a pivotal role in our freshwater future.

By Dr. Sally Entrekin
Department of Biology, University of Central Arkansas

Dr. Entrekin is an environmental scientist; she earned her BS in Biology at Georgia Southwestern University, and MS in Entomology from the University of Georgia, and a PhD in Biology from the University of Notre Dame. Dr. Entrekin has been a faculty member at UCA since 2008. The basic question addressed by Dr. Entrkin's lab is: "How do natural and human disturbances interact to influence aquatic community composition and function." Dr. Entrekin is widely pub-lished and has made many presentations at national and regional meetings. Recent work has included stream ecology in the area of the Fayetteville Shale. This work includes examining how rapid land use changes, such as natural gas extraction and urbanization, alter associated freshwater ecosystems, and how interactions among common and emerging watershed activities change stream structure and functions.

SECTION PROGRAMS
ORAL PRESENTATIONS
(Only the presenter's name was available at time of printing)

ORAL SESSIONS: FRIDAY 1:00-5:30

BIOLOGICAL SCIENCES - MEDICINE AND MICROBIOLOGY ROOM CCCS 207
Chair: A. Mukherjee

1:00
COLLAGEN INCREASES TUMORIGENICITY OF PAPILLARY THYROID CANCER CELLS HARBORING BRAFV600E MUTATIONS
Anna Sharabura. Hendrix College

1:15
FOCAL ADHESION KINASE DISPLAYS ALTERED REGULATION IN PAPILLARY THYROID CANCER
Ben Zamzow. Hendrix College

1:30
THE USE OF LIGATION INDEPENDENT CLONING TO GENERATE ESCHERICHIA COLI CAPABLE OF PRODUCING CENTRUROIDES VITTATUS SCORPION ÊŽμ-TOXINS NA681 AND NA682 TO ALLOW FOR FURTHER ANALYSIS OF PHYSIOLOGICAL AND MEDICAL SIGNIFICANCE
David Williams. Arkansas Tech University

1:45
VALIDATION OF METHODS TO IMPROVE THE USE OF P19 CELLS AS A MODEL OF NEURONAL DIFFERENTIATION
Wallace Williamson. Arkansas Tech University

2:00
RELATIVE GENE EXPRESSION STUDY ON CENTRUROIDES VITTATUS INVESTIGATING SODIUM TOXIN GENE ACTIVITY
Aimee Bowman. Arkansas Tech University

2:15
USING ESCHERICHIA COLI TO GENERATE SCORPION Î2-TOXINS, AS SEEN IN CENTRUROIDES VITTATUS, TO ALLOW FOR FURTHER STRUCTURAL AND PHYSIOLOGICAL ANALYSIS
Jacob Pinkerton. Arkansas Tech University

2:30
MITOCHONDRIA MORPHOLOGY AND ROTENONE
Hunter Scharbor. University of Central Arkansas

2:45
OBSERVING FSZ-B'S FUNCTION IN DICTYOSTELIUM DISCOIDEUM MITOCHONDRIAL DYNAMICS
Pristine Pittman. University of Central Arkansas

CHEMISTRY ROOM CCCS 115
Chair: A. Harrington

1:00
FLUORESCENT INHIBITOR OF SYSTEM Xc-
Alan Jackson. Arkansas Tech University

1:15
BOND LENGTH AND BOND VALENCE FOR TUNGSTEN - OXYGEN AND TUNGSTEN - SULFUR BONDS
Ruth Lykins. Arkansas Tech University

1:30
ATMOSPHERIC PHOTOCHEMISTRY STUDIES RELATED TO SATURN'S MOON TITAN
Connor Purvis. Harding University

1:45
ANALYSIS OF SURFACE WATERS IN SEARCY, ARKANSAS
Jade Toth. Harding University

2:00
BOND LENGTH AND BOND VALENCE RELATIONSHIP OF CHROMIUM OXIDES, CHROMIUM SULFIDES, MOLYBDENUM OXIDES, AND MOLYBDENUM SULFIDES
Jordan Labrecque. Arkansas Tech University

2:15
PHOTOCATALYTIC STERILIZATION OF AQUEOUS SOLUTIONS
David Williams. Arkansas Tech University

2:30
CARBON-CARBON, CARBON-OXYGEN, CARBON-NITROGEN, AND CARBON-HYDROGEN BOND VALENCE -LENGTH RELATIONSHIPS
Franklin Hardcastle. Arkansas Tech University

2:45
SEARCHING FOR PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN WASTEWATER IN SEARCY, ARKANSAS
Natalie Whitlock. Harding University

PHYSICS ROOM CCCS 101
Chair: M. Hemmati

1:00
SUPPRESSION OF RADIATION-INDUCED CHROMOSOME DAMAGE BY GT3 AND THE ROLE OF MICROGRAVITY
Calla Bassett. Southern Arkansas University

1:15
A SIGN-CHANGING INTERACTION BETWEEN DARK ENERGY AND MATTER
Jazmin Urioste. Arkansas Tech University

1:30
IONIZATION RATE FOR BREAKDOWN WAVES WITH A SIGNIFICANT CURRENT BEHIND THE WAVE FRONT
Jesse Griffiths. Arkansas Tech University

1:45
OPTICAL AND DISPERSION PARAMETERS OF PMMA DOPED BY INDIUM SALT
Sami Salman Chiad. Al-Mustansaryah University

2:00
DESIGN OF CENTRAL RECEIVER SOLAR THERMAL CONCENTRATOR
Alaa H. Shneishil. Al-Mustansaryah University

2:15
A NEW RELATION BETWEEN SPIRAL ARM PITCH ANGLES (P) AND THE BULGE LUMINOSITY
Ismaeel Al-Baidhany. Al-Mustansaryah University

BIOLOGICAL SCIENCES AQUATICS
ROOM CCCS 105
Chair: A. Odendaal

1:00
SPATIO-TEMPORAL DYNAMICS OF FISH ASSEMBLAGE STRUCTURE AFTER WATERSHED ALTERATION IN THE SALINE RIVER, ARKANSAS
Aaron Burgad. University of Central Arkansas

1:15
ENVIRONMENTAL DNA VS. TRADITIONAL SAMPLING: A CASE STUDY USING THE FEDERALLY THREATENED LEOPARD DARTER, *PERCINA PANTHERINA*
Taylor Lee. Arkansas State University

1:30
GENETIC STRUCTURE AND DIVERSITY OF DISJUNCT POPULATIONS OF RAINBOW DARTERS (*ETHEOSTOMA CAERULEUM*) AND SOUTHERN REDBELLY DACE (*CHROSOMUS ERYTHROGASTER*) THROUGHOUT THE MISSISSIPPI CORRIDOR.
Kyle Dineen. Arkansas State University

1:45
HISTORIC CHANGES IN FISH ASSEMBLAGE PATTERNS IN THE LITTLE MISSOURI RIVER, ARKANSAS
Michelle Furr. University of Central Arkansas

2:00
SPATIOTEMPORAL POPULATION DYNAMICS OF THE CADDO MADTOM (*NOTURUS TAYLORI*)
Brittany McCall. Arkansas State University

2:15
FISH-HABITAT ASSOCIATIONS IN THE KINGS RIVER, ARKANSAS
Chelsey Sherwood. University of Central Arkansas

2:30
THE ANALYSIS OF COLIFORM BACTERIA IN LAKE COLUMBIA, AR.
Kara O'Neal. Southern Arkansas University

2:45
COMPONENTS OF URBANIZATION AND URBAN PROXIMITY IDENTIFY THREATS TO STREAM WATER QUALITY
Stephanie Stoughton. University of Central Arkansas

BIOLOGICAL SCIENCES – GENERAL
ROOM CCCS 207
Chair: J. Kelly

3:30
DISEASE INTRODUCTION BY ABORIGINAL HUMANS IN NORTH AMERICA AND THE PLEISTOCENE EXTINCTION
Zachary Nickell. Hendrix College

3:45
THE USE OF COLOR AS A STATUS SIGNAL IN THE PRAIRIE LIZARD, *SCELOPORUS CONSOBRINUS*
Christopher Robinson. University of Central Arkansas

4:00
WHO CITES WHOM? COMMUNICATION INSIGHTS FROM A BIBLIOMETRIC ANALYSIS OF INVASION AND BIOLOGICAL CONTROL LITERATURE
Ashley Schulz. Arkansas State University

4:15
ECOSYSTEM SERVICES OF THE BIG BEND REGION OF THE CHIHUAHUAN DESERT
Helena Abad. Hendrix College

4:30
BIODIVERSITY IN BIOCUBES
Amanda Brooks. Ouachita Baptist University

4:45
A FRACKING RACKET: DO BIRDS CHANGE THE WAY THEY SING WHEN EXPERIENCING CHRONIC NOISE FROM SHALE GAS EXTRACTION
Claire Turkal. Harding University

ENGINEERING & COMPUTER SCIENCE
ROOM CCCS 115
Chair: C. Frederickson

3:30
GENERATIVE ANATOMY MODELLING LANGUAGE (GAML)
Doga Demirel. University of Arkansas at Little Rock

3:45
COMPUTING WIND FIELD CLOSE TO THE GROUND USING CFD IN A TORNADO CHAMBER.
Damaso Dominguez. University of Arkansas

4:00
SIMULATING FOODBORNE PATHOGENS IN POULTRY PRODUCTION AND PROCESSING TO DEFEND AGAINST INTENTIONAL CONTAMINATION
Silas Lankford. University of Arkansas

4:15
CALCULATION OF THE WIDTH AND PERIOD OF TORNADOS FROM GOOGLE EARTH DATA AND THERELATIONSHIP BETWEEN THE TORNADO'S PERIOD AND THE WIND SPEED
Mohammadhossein Kashefizadeh. University of Arkansas

4:30
NANOSTRUCTURES FOR INFRARED LINEAR POLARIZERS
Marzia Zaman. University of Arkansas

4:45
AN ACOUSTIC-BASED APPROACH FOR CONDITION MONITORING OF PIPES
Mitchell Collins. Arkansas Tech University

BIOLOGICAL SCIENCES- INVERTEBRATES
ROOM CCCS 105
Chair: D. Barron

3:30
A DESCRIPTION OF VARIATION IN FECUNDITY BETWEEN TWO POPULATIONS OF WOLF SPIDER *RABIDOSA RABIDA* IN SEARCY ARKANSAS USING BROOD SIZE MEASUREMENTS
Brandon Hogland. Harding University

3:45
SEASONAL CHANGES OF ARTHROPOD COMMUNITY STRUCTURE IN INACTIVE BISON WALLOWS
Sofia Varriano. Hendrix College

4:00
A FIRST LOOK INTO THE MICROBIOME OF *RABIDOSA RABIDA*, A WOLF SPIDER IN SEARCY, ARKANSAS
Patricia Rivera. Harding University

4:15
MIGRATION DYNAMICS OF OHIO SHRIMP, MACROBRACHIUM OHIONE, IN ARKANSAS
Geoffry Spooner. University of Central Arkansas

4:30
DISTRIBUTION, HABITAT, AND LIFE HISTORY ASPECTS OF THE SHRIMP CRAYFISH, *FAXONIUS LANCIFER* (HAGEN) (DECAPODA: CAMBARIDAE) IN ARKANSAS
Chris McAllister. Eastern Oklahoma State College-Idabel

4:45
CRAYFISH CHANGES TO THE ARKANSAS WILDLIFE ACTION PLAN
Brian Wagner. Arkansas Game and Fish Commission

BIOLOGICAL SCIENCES- BOTANY
ROOM CCCS 211
Chair: A. Willyard

3:30
TRANSCRIPTOME ANALYSIS FOR THREE *ERIGERON* (ASTERACEAE) GENOTYPES DIFFERING IN MODE OF REPRODUCTION
James Vire. University of Central Arkansas

3:45
MITOCHONDRIAL LINEAGES OF *PINUS* SUBSECTION PONDEROSAE TO RESOLVE THE RELATIONSHIP OF SPECIES NAMED IN THE UNITED STATES AND MEXICO
Mason Sifford. Hendrix College

4:00
DETERMINING THE VALIDITY OF THE SPECIES *PINUS WASHOENIS* IN THE WESTERN UNITED STATES
Samuel Lockhart. Hendrix College

4:15
DETERMINING RECENT HYBRIDIZATION OF PINYON PINES IN A ZONE OF SYMPATRY IN NORTHERN ARIZONA
Katie Dobbins. Hendrix College

4:30
SEED DISPERSAL OF DIOSPYROS VIRGINIANA IN THE PAST AND THE PRESENT: EVIDENCE FOR A GENERALIST EVOLUTIONARY STRATEGY
Taylor Stone. Hendrix College

4:45
SEED DISPERSAL IN OSAGE ORANGE (*MACLURA POMIFERA*) BY SQUIRRELS (*SCIURUS* SPP.)
Sophie Katz. Hendrix College

GEOSCIENCE ROOM CCCS 101
Chair: J. Allender

3:30
LATE MISSISSIPPIAN (CHESTERIAN) SHALLOWING-UPWARD, EUSTATIC CYCLICITY REFLECTED BY TAPHONOMY OF PRESERVED AMMONOID CEPHALOPODS, NORTHERN ARKANSAS
Riley Dickson. University of Arkansas, Fayetteville

3:45
LITHOLOGIC CHARACTER, SEQUENCE AND DIAGENETIC HISTORY OF LOWER MISSISSIPPIAN TRIPOLITIIC CHERT, NORTHERN ARKANSAS AND SOUTHERN MISSOURI
Sydney McKim. University of Arkansas, Fayetteville

4:00
LOWER MISSISSIPPIAN, TRIPOLITIC CHERT FORMED FROM HYDROTHERMALLY EMPLACED SILICA, AND ITS POSSIBLE RELATIONSHIP TO THE TRI-STATE LEAD-ZINC MINING DISTRICT
Jonathan Chick. University of Arkansas, Fayetteville

4:15
ESTABLISHING A TRAIL VISITATION SURVEY METHOD FOR THE NATIONAL OUACHITA RECREATION TRAIL
Emily Roberts. University of Central Arkansas

4:30
TECTONO-STRATIGRAPHIC AND SEQUENCE AND STRATIGRAPHIC SUCCESSIONS, OZARK SHELF, TRI-STATE REGION, SOUTHERN MIDCONTINENT.
Elvis Bello. University of Arkansas, Fayetteville

4:45
SIGNIFICANCE OF THE MIDDLE MISSISSIPPIAN PALEOKARST SURFACE, WESTERN OZARK UPLIFT, ARKANSAS, KANSAS AND OKLAHOMA
Walter Manger. University of Arkansas, Fayetteville

ORAL SESSIONS: SATURDAY 8:00-10:15

BIOLOGICAL SCIENCES ROOM CCCS 105
Chair: D. James

8:00
COMPARISON OF BACTERIAL COMMUNITIES IN NATURAL AND DEVELOPED WATERSHEDS OF THE BUFFALO NATIONAL RIVER USING CULTIVATION AND 16S METAGENOMICS TECHNIQUES
Michael Ukpong. North Arkansas College

8:15
PERSISTENCE OF DOWNSTREAM NEGATIVE IMPACT OF POINT SOURCE AND NON-POINT SOURCE STREAM POLLUTANTS
Timothy Wakefield. John Brown University

8:30
HISTOLOGY OF RATHKE'S GLANDS IN THE RAZOR-BACKED MUSK TURTLE, *STERNOTHERUS CARINATUS* (CHELONIA: KINOSTERNIDAE), WITH COMMENTS ON LAMELLAR BODIES
Stan Trauth. Arkansas State University

8:45
COCCIDIAN PARASITES (APICOMPLEXA: EIMERIIDAE) OF ARKANSAS HERPETOFAUNA: A SUMMARY WITH TWO NEW STATE RECORDS
Chris McAllister. Eastern Oklahoma State College-Idabel

9:00
ECOLOGY OF BLANCHARD SPRINGS CAVERNS, OZARK NATIONAL FOREST, ARKANSAS, 42 YEARS LATER
Selena Sasser. Southern Illinois University

9:15
VERTEBRATE NATURAL HISTORY NOTES FROM ARKANSAS, 2017
Renn Tumlison. Henderson State University

9:30
BAT OCCUPANCY ESTIMATES AND SPECIES RICHNESS AT CACHE RIVER NATIONAL WILDLIFE REFUGE
Virginie Rolland. Arkansas State University

9:45
RESULTS OF TRAPPING SMALL MAMMAL POPULATIONS IN A GRASSLAND AND FOREST AREA AT THE LAKE FAYETTEVILLE ENVIRONMENTAL CENTER.
Douglas James. University of Arkansas

10:00
SULFUR OXIDATION GENOMICS IN THE AEROBIC PURPLE SULFUR BACTERIUM *HALOTHIOBACILLUS NEAPOLITANUS*
Newton Hillard. Arkansas Tech University

ENGINEERING/CHEMISTRY ROOM CCCS 115
Chair: M. Khan

8:00
DEVELOPING A LOW COST 3D PRINTING LAB AND THE USE OF 3D PRINTING IN A FRESHMAN ENGINEERING LAB COURSE
Mahbub Ahmed. Southern Arkansas University

8:15
RESTRAINED SHRINKAGE OF FLY ASH BASED GEOPOLYMER CONCRETE AND ANALYSIS OF LONG TERM SHRINKAGE PREDICTION MODELS
Md Rashedul Islam. Southern Arkansas University

8:30
OPTICAL PROPERTIES OF IRRADIATED SNO_2 THIN FILMS PREPARED BY CHEMICAL SPRAY PYROLYSIS
Ali N. Mohammed. Al-Mustansaryah University

8:45
SWEETER SCORPIONATES INCORPORATE CARBOHYDRATES INTO FUNCTIONAL METAL CHELATES
Patrick Desrochers. University of Central Arkansas

9:00
DESIGN AND SYNTHESIS OF NIR DONOR-ACCEPTOR FLUOROPHORES
Rajib Choudhury. Arkansas Tech University

BIOLOGICAL SCIENCES ROOM CCCS 101
Chair: T. Yamashita

8:00
GEOGRAPHIC DISTRIBUTION OF YELLOW GRUB IN SMALLMOUTH BASS POPULATIONS OF CROOKED

CREEK AS DETERMINED BY METACERCARIAL CYST COUNTS IN THE GILL-MOUTH (OROBRANCHIAL) SITES
James Daly Sr. University of Arkansas for Medical Sciences (retired)

8:15
THE FISHES OF CHADRON CREEK, DAWES COUNTY, NEBRASKA
Chris McAllister. Eastern Oklahoma State College-Idabel

8:30
ANATOMICAL DISTRIBUTION OF *CLINOSTOMUM* METACERCARIAE IN THE TISSUES OF POND-RAISED CHANNEL CATFISH
James Daly Sr. University of Arkansas for Medical Sciences (retired)

8:45
PHYLOGEOGRAPHY AND VICARIANT SEPARATION OF TWO RIVER DARTERS, *PERCINA URANIDEA* AND *PERCINA VIGIL*, FROM THE NORTH AMERICAN INTERIOR HIGHLANDS
Tsunemi Yamashita. Arkansas Tech University

9:00
LONG-TERM AQUATIC INVERTEBRATE MONITORING AT BUFFALO NATIONAL RIVER, ARKANSAS
David Bowles. US National Park Services

9:15
DEVELOPMENT OF THE ARKANSAS CENTER FOR BIODIVERSITY COLLECTIONS (ACBC) AT ARKANSAS STATE UNIVERSITY
Brook Fluker. Arkansas State University

9:30
LIRIOPE AND OPHIOPOGON: OVERVIEW OF TWO GENERA OF RUSCACEAE NATURALIZED IN THE ARKANSAS FLORA
Megan Stone. Henderson State University

9:45
NEW AND NOTEWORTHY VASCULAR PLANT RECORDS FROM ARKANSAS
Brook Olsen. Henderson State University

10:00
LEECH PARASITISM OF THE GULF COAST BOX TURTLE, *TERRAPENE CAROLINA MAJOR* (AGASSIZ, 1857) (TESTUDINES: EMYDIDAE)
Dennis Richardson. Quinnipiac University

POSTER PRESENTATIONS

BIOLOGICAL SCIENCES POSTER PRESENTATIONS

1. **THE TOXICOLOGICAL EFFECTS OF DIETARY SUPPLEMENT ADDITIVES ON *DAPHNIA MAGNA*.**
   Antoinette Odendaal

2. **SUNBATHING IN THE NORTHERN ROADRUNNER: AN UNEXPLORED TRAIT FOR THEIR RANGE EXTENSION.**
   Kimberly Smith

3. **LITERATURE RECORD CHECKLIST OF TRUE BUGS (HEMIPTERA) FOR ARKANSAS WITH THE FIRST REPORT OF *PSEUDOPACHYBRACHIUS VINCTUS* (RHYPAROCHROMIDAE) (ALSO OKLAHOMA) AND AN ANALYSIS OF MIRIDAE SEX RATIO FROM LIGHT TRAPS.** Stephen Chordas III

4. **SURVEY OF RODENTS WITHIN ARKANSAS GAME AND FISH COMMISSION WILDLIFE MANAGEMENT AREAS.**
   Matthew Connior

5. **NEW RECORDS OF PARASITES (APICOMPLEXA, ACARI, ANOPLURA, NEMATODA) FROM RODENTS IN ARKANSAS.**
   Matthew Connior

6. **DISTRIBUTION OF THE EASTERN SPOTTED SKUNK, *SPILOGALE PUTORIUS*, IN THE EARLY TWENTIETH CENTURY.**
   D. Blake Sasse

7. **CENTRAL NESTS ARE MORE SUCCESSFUL AND PREFERRED FOR REUSE THAN PERIPHERAL NESTS IN CLIFF SWALLOW (*PETROCHELIDON PYRROHONOTA*) COLONIES.**
   Steward Huang

8. **THE FLEAS (ARTHROPODA: INSECTA: SIPHONAPTERA) OF ARKANSAS.**
   Matthew Connior

9. **AN ANNOTATED CHECKLIST OF THE CRAYFISHES (DECAPODA: CAMBARIDAE) OF ARKANSAS.**
   Chris McAllister

10. **UNDERSTANDING CONFRONTATIONAL BEHAVIOR OF THE RAINBOW LORIKEET (*TRICHOGLOSSUS HAEMATODUS*) AS A STRATEGY TO REDUCE ITS IMPACT AS AN INVASIVE SPECIES.**
    Victoria Veerhusen

11. **PHYSIOLOGICAL EFFECTS OF LEAD NITRATE ON FOUR ARKANSAS NATIVE PLANT SPECIES AND THE ROLE OF ARBUSCULAR MYCORRHIZAL FUNGI ON PLANT STRESS MITIGATION.**
    Nicholas Dial

12. **THE EFFECTS OF MICROGRAVITY ON VASCULAR TONE IN FEMALE MICE.**
    Sage Shaddox

13. **ANALYSIS OF THE OXIDATIVE ENZYMATIC PROPERTIES OF THE BOTTLENOSE DOLPHIN DIAPHRAGM AND SCALENUS MUSCLE.**
    McKenzie Stribling

14. **A QUALITATIVE ANALYSIS OF MACROINVERTEBRATES IN THE UCA VERNAL PONDS.**
DeShauna Tucker

15. **SWIMMING PREFERENCES OF GUINEA PIGS.**
Olivia Sims

16. **THE EFFECT OF HABITAT FRAGMENTATION ON *SCELOPORUS CONSOBRINUS*-PRAIRE LIZARDS LOCATED IN CENTRAL ARKANSAS.**
Mallory Heft

17. **A HABITAT SUITABILITY ANALYSIS OF THE RED WOLF ACROSS ITS HISTORIC RANGE.**
Lauren Toivonen

18. **EFFECT OF STYROFOAM DIET ON THE GUT PROTISTS OF MEALWORMS (*TENEBRIO MOLITOR*).**
Brooke Skinner

19. **LONGITUDINAL PATTERNS IN AN ARKANSAS RIVER VALLEY STREAM: TESTING THE RIVER CONTINUUM CONCEPT.**
Angela Lenard

20. **UTILITY OF THE LOW COPY NUCLEAR GENE LEAFY FOR PHYLOGENETIC STUDIES IN THE SUNFLOWER FAMILY (ASTERACEAE), TRIBE ASTEREAE.**
Miguel Mercado

21. **RWANDAN SOCIETAL IMPROVEMENTS VIA THE USE OF ROCKET STOVES.**
Kyle Davis

22. **ENZYME PRODUCTION AND HORMONE SIGNALING MEDIATE LONGEVITY RESPONSES TO DIETARY RESTRICTION IN *C. ELEGANS*.**
Ashley Henderson

23. **ISOLATION OF ERYTHROCYTES FROM FRESHWATER TURTLES FOR USE IN TELOMERE LENGTH ANALYSIS.**
EliasSmith

24. **INFLUENCE OF COMMON SALT CONCENTRATIONS ON DETRITIVORE RESPIRATION.**
Billy Huggins

25. **BODY SHAPE VARIATION WITHIN AND AMONG LINEAGES OF THE RAINBOW DARTER, *ETHEOSTOMA CAERULEUM*.**
Kandria Driskill

26. **EFFECTS OF BLACK-SPOT DISEASE ON THE BODY CONDITION OF BLEEDING SHINERS, *LUXILUS ZONATUS*.**
Lindsey Martin

27. **BIOREMEDIATION OF PETROCHEMICALS FROM SOIL.**
Mitchell Rowland

28. **WESTERN SPRUCE BUDWORM HERBIVORY INFLUENCES STREAM MACROINVERTEBRATE STRUCTURE AND BIOMASS.**
Deion Everhart

29. **MACROINVERTEBRATE COMMUNITY RESPONSE TO A GRADIENT OF URBANIZATION IN THE WHITE OAK BAYOU WATERSHED.**
Joshua Nilz

30. **PREY ITEMS IMPALED BY LOGGERHEAD SHRIKES IN NORTHWESTERN ARKANSAS.**
Ross Shiery

31. **IMMUNOLOCALIZATION OF THE CADHERIN 18 PROTEIN IN FETAL KIDNEY CELLS.**
Scotty McKay

32. **ANALYSIS OF BODY SHAPE VARIATION AMONG RESTRICTED AND WIDESPREAD POPULATIONS OF THE SOUTHERN REDBELLY DACE, *CHROSOMUS ERYTHROGASTER*.**
Dustin Thomas

33. **RECRUITMENT AND SURVIVAL IN *LIGUSTRUM SINENSE*.**
Mason Rostollan

34. **ANALYSIS OF A *DROSOPHILA MELANOGASTER* RIBOSOMAL PROTEIN GENE.**
John Mitchell

35. **EVALUATING THE EFFECTS OF BIRD FEEDERS ON SONGBIRD PLUMAGE COLORATION.**
Stetson R. Collard

36. **SOIL CRUST ALGAL COMMUNITIES OF WARREN PRAIRIE NATURAL AREA.**
Crystal Haynes

37. **A COMPARISON OF THE IMPACTS OF WIND ENERGY AND UNCONVENTIONAL GAS DEVELOPMENT ON LAND-USE AND ECOSYSTEM SERVICES: AN EXAMPLE FROM THE ANADARKO BASIN OF OKLAHOMA, USA.**
Kendall Davis

38. **LAND-USE AND ECOSYSTEM SERVICES COSTS OF UNCONVENTIONAL OIL AND GAS IN THE U.S.**
Nathan Taylor

39. **IMPACT OF BAKKEN/THREE FORKS UNCONVENTIONAL OIL AND GAS DEVELOPMENT ON IMPORTANT WILDLIFE LANDSCAPES IN NORTH DAKOTA.**
Howden Carly

40. **OPTIMUM LIGHTING FOR OXYGEN PRODUCTION OF ARTHROSPIRA PLATENSIS.**
Camryn Ruggeri

41. **CHARACTERIZING A PUTATIVE MYCO-BACTERIOPHAGE TOXIN-ANTITOXIN SYSTEM.**
Alex Abbott

42. **EXPRESSION OF OCT4 AND NANOG IN HUMAN FIBROBLASTS WITH SHORT TELOMERES.**
Ethan Clement

43. **CHARACTERIZING THE PERSISTING CORTICAL SUBPLATE IN RATS ACROSS DEVELOPMENT, MATURATION, AND AGING.**
Terri Teague

44. **GLUCOSE-MEDIATED NEUROPROTECTION AGAINST PROTEOTOXICITY IS ENERGY-DEPENDENT IN *CAENORHABDITIS ELEGANS*.**
Landon Gatrell

45. **THE EFFECTS OF TRPC3 CHANNEL ACTIVATION ON VASCULAR TONE IN HEALTHY ARTERIES VERSUS DISEASED ARTERIES.**
Alan Jackson.

46. **COMPARATIVE GENOMICS OF THE PARABURKHOLDERIA BACTERIAL SYMBIONTS OF THE SOCIAL AMOEBA *DICTYOSTELIUM DISCOIDEUM*.**
Alexandria Melton

47. **EPIDEMIOLOGY OF PANDEMIC INFLUENZA IN ARIZONA.**
Jenna Suen

48. **EPIDEMIOLOGY OF PANDEMIC INFLUENZA IN MISSOURI.**
Harleigh Robbins

49. **LIPID EXTRACTION OF C. *ELEGANS*.**
Lauren Westwood

50. ***CAENORHABDITIS ELEGANS* HERMAPHRODITES HAVE REDUCED STRESS TOLERANCE WHEN GROWN IN THE PRESENCE OF RESIDUAL MALE PHEROMONES.**
Nathan Carmichael

51. **GLUCOSE MEDIATES EARLY-LIFE STRESS RESISTANCE IN *C. ELEGANS*.**
Kaitlynn Butler

52. **REGULATION OF SYMBIOTIC GENE EXPRESSION IN RICE.**
Ha Ram Kim

53. **THE INFLUENCE OF EMBRYONIC STRESS HORMONES ON PHENOTYPIC VARIATION AND FITNESS IN *SCELOPORUS CONSOBRINUS***
Marcia Polett

CHEMISTRY POSTER PRESENTATIONS

54. **SYNTHESIS OF AMINO ACID RADICAL PRECURSORS.**
Candice Foscue

55. **RETINOIC ACID RECEPTOR GAMMA RECIPROCALLY REGULATES CELLULAR ADHESION AND PROLIFERATION IN K562 CELLS.**
Victoria Niedzwiedz

56. **IMPACT OF CHEMISTRY/MATHEMATICAL VIDEO-LECTURES ON STUDENT-BASED LEARNING IN A FRESHMAN LEVEL CHEMISTRY COURSE.**
Aaron Stebbins

57. **ANALYZING THE PROPERTIES OF BIODIESEL.**
Dakotah Cooper

58. **IDENTIFYING THE STRUCTURE OF FAT-MOBILIZING SUBSTANCE (FMS-1) ASSOCIATED WITH CONGENITAL LIPODYSTROPHY.**
Mallory Bryant

59. **MEASUREMENT OF MULTICOMPONENT AEROSOL OPTICAL PROPERTIES USING PULSED LASER CAVITY RING-DOWN SPECTROSCOPY.**
Haley Kay

60. **THEORETICAL SEMI-EMPIRICAL CALCULATIONS OF ETHYLENEDIAMINETETRAACETIC ACID (EDTA) BOUND METAL COMPLEXES MODELED FOR HEAVY METAL POISONING.**
John Bentley

61. **THEORETICAL THERMOCHEMICAL BOND STABILITY OF EDTA CHELATION WITH METAL IONS FOR CHELATION THERAPY TO TREAT HEAVY METAL POISONING.**
Peter Joseph

62. **DETERMINATION OF FATTY ACID CONCENTRATIONS IN ALGAE.**
Stacy Justice

63. **ALTERING THE CONFORMATIONAL ENSEMBLE OF PEP-19 USING CALCIUM.**
Nicholas Rathke

64. ***IN SILICO* PREDICTIONS OF CONFORMATIONS AND ORIENTATIONS OF SMALL MOLECULES WITHIN A MACROCYCLE.**
Kassandra Cendejas

65. **THE EFFECT OF VIDEO-BASED INSTRUCTION ON STUDENT PERFORMANCE IN A COLLEGE CHEMISTRY LABORATORY.**
Kaitlyn Walden

66. **CONFORMATIONAL PROTEIN ENSEMBLE OF THE CALMODULIN LIGAND PEP-19.**
Danica Ordonez

67. **EXPRESSION AND PURIFICATION OF CALMODULIN BINDING PARTNER PEP-19.**
Christian Mitchell

68. **WATER ADSORPTION ON MONTMORILLONITE: COMPARISON OF WASHED AND UNWASHED CLAYS.**
Megan Cassingham

69. **THE EFFECT OF SIMULATED MICROGRAVITY ON THE BIOPHYSICAL PROPERTIES OF THE TIBIA.**
Kristen Jones

70. **TOWARDS A CHLORINE-BASED FLUOROPHORE SYNTHESIS FOR PHARMACOKINETIC DIAGNOSIS IN NEGLECTED TROPICAL DISEASES.**
Shelby Bridges

71. **THE SYNTHESIS OF NATURAL PRODUCT-BASED DRUGS TO TREAT A NEGLECTED TROPICAL DISEASE: LEISHMANIASIS**.
Adam Hargis

COMPUTER SCIENCE POSTER PRESENTATIONS

72. **DESIGN OF VIRTUAL ENDOLUMINAL SURGERY SIMULATOR (VESS).**
Berk Cetinsaya

73. **VIRTUAL ARTHROSCOPIC TEAR DIAGNOSIS AND EVALUATION PLATFORM (VATDEP).**
Mustafa Tunc

74. **A GENTLENESS SIMULATOR FOR SURGICAL DEXTERITY EVALUATION OF SURGEONS WITH HAPTIC INTERFACES.**
Recep Erol

75. **ANALYSIS AND COMPARISON OF WINE EXPERTS WITH WINEINFORMATICS.**
Valentin Velchev

76. **DRONE DELIVERY WITH A PARROT BEBOP 2.**
James Palmer

77. **LOW-DELAY RATE CONTROL FOR H.265/HEVC VIDEO COMPRESSION.**
Reese Childers

78. **ORION ROVER MAZE SOLVER AND OBJECT DETECTION.**
Binqi Sun

79. **EDUCATIONAL DRONES: MINI-DRONE TRAVIS BY PARROT.**
Sravani Marreddy

80. **ARTHROSCOPIC ROTATOR CUFF SURGERY PERFORMANCE COMPARISON STUDY OF NOVICES AND EXPERTS.**
Seth Cooper-Baer

81. **JD HUMANOID ROBOT.**
Min Kim

ENGINEERING POSTER PRESENTATIONS

82. **EVALUATION OF A 3D METAL-ORGANIC FRAMEWORK $Cu(bpy)V_4O_{10}$ AS A CATHODE MATERIAL FOR Li-ION BATTERIES.**
Andrew Zhou

83. **TERAHERTZ PULSED IMAGING OF FRESHLY EXCISED XENOGRAFT BREAST CANCER TUMORS IN MICE.**
Tyler Bowman

84. **RADIATION PRESSURE ON CORE-SHELL NANOPARTICLES.**
Mohammad Habibur Rahaman

85. **ANALYTICAL MODELING OF MICROSCOPIC AND MACROSCOPIC ANALYSIS OF DIELECTRIC MATERIAL.**
Misuk Saha

86. **IMAGE ANALYSIS TOOLBOXES FOR FINDING THE MEAN PORE SIZE OF SCAFFOLDS AND DIAMETER DISTRIBUTION OF FIBERS.**
Samia Sanjari

GEOSCIENCE POSTER PRESENTATIONS

87. **TECTONO-STRATIGRAPHIC AND SEQUENCE AND STRATIGRAPHIC SUCCESSIONS, OZARK SHELF, TRI-STATE REGION, SOUTHERN MIDCONTINENT.**
Elvis Bello

88. **UTILIZING KARST FEATURES TO ACCOUNT FOR DIFFERENCES IN WATER QUALITY IN THE OZARK PLATEAUS IN NORTHERN ARKANSAS.**
Samantha Wacaster

89. **BRIDGING TECHNOLOGY AND COMMUNITY USING WEB-BASED GIS.**
Samuel Treece

90. **WATER ADSORPTION ON GOETHITE: CCN ACTIVATION FROM FRENKEL, HALSEY AND HILL ACTIVATION THEORY.**
Ryan Tumminello

91. **QUANTIFICATION OF LEVOGLUCOSAN IN PM2.5 ATMOSPHERIC AEROSOLS USING GC/MS: SEASONALITY OF A BIOMASS BURNING TRACER IN CENTRAL ARKANSAS.**
Abigail Gatmaitan

PHYSICS POSTER PRESENTATIONS

92. **GENERATION OF MIXED-PARITY INCE-GAUSS LASER BEAMS.**
Jessica Young

93. **THE IMPACT OF WEATHER ON MUSIC FESTIVALS IN OKLAHOMA**
Qinqing Yang

94. **OPTIMAL LOCATIONS FOR THE GENERATION OF RENEWABLE ENERGY.**
Justin Seymour

95. **CREATION OF IMAGE MASKS FOR BARRED SPIRAL GALAXIES USING SOURCE EXTRACTOR.**
Jess Ray

96. **POTENTIAL DIFFERENCES IN PROBLEM SOLVING APPROACHES WHEN USING DIFFERENT TEXTBOOKS.**
Charles Bertram

97. **PROCESS ENGINEERING: 3 DIMENSIONAL COLORSPACE CORRECTION.**
Joseph Akers

98. **DESIGN AND DEVELOPMENT OF AN ACOUSTIC FIELD SCANNER.**
Nick Scoles

99. **THE LAGRANGIAN AND HAMILTONIAN FORMULATION OF THE LINEAR OSCILLATOR CHAIN.**
Garrott Granholm

100. **MAXIMIZING WIND POWER.**
Royer Ramirez Ruiz

101. **THE EFFECTS OF WEATHER ON INSURANCE.**
ZaneAkers

102. **DETERMINING BLACK HOLE MASS OF ACTIVE GALACTIC NUCLEI USING FWHM OF THE H-Î² EMISSION LINE AND LUMINOSITY RELATIONS.**
Jacob Cameron

103. **A GEOMETRIC APPROACH TO DETERMINING CONSERVATION LAWS FOR WAVES ON A STRING.**
Brandon Miller

104. **MAPPING THE INFLUENCE OF WEATHER ON CONCRETE.**
Nick Scoles

105. **THE EFFECTS OF WEATHER ON INSURANCE.**
Joseph Akers

106. **PRECIPITATION TOTALS AND THE EFFECT ON SOIL TEMPERATURE.**
Timothy Brown

107. **MECHANICAL AND CHEMICAL ALTERATIONS IN RAT LEG BONES UPON EXPOSURE TO SIMULATED MICROGRAVITY AND COSMIC RADIATION.**
Hayley Heacox

108. **RENEWABLE ENERGY IN RESEARCH AND EDUCATION.**
Lauren Corby

109. **TOMATO GROWTH IN OKLAHOMA.**
Audrey Schucker

110. **IMPACT OF MESONET ON OKLAHOMA AGRICULTURAL PROCESSES.**
Isaac Raphael

111. **AIR TEMPERATURE/ DEW POINT VS. FREQUENCIES OF TORNADOES.**
Garrott Granholm

112. **CORRELATION OF AVERAGE TEMPERATURE VS. CONSECUTIVE TEMPERATURE TO DROUGHT CONDITIONS IN THE OKLAHOMA CITY AREA.**
Blake Bryant

113. **OPTIMAL CROP GROWING SEASONS DETERMINED USING MESONET.**
EliasSmith

114. **NEUTRON CAPTURE ELEMENTS IN LOW METALLICITY STARS WITHIN THE INNER-GALACTIC HALO.**
Kenneth Jumper

115. **THE EFFECT OF MODEL ATMOSPHERE PARAMETERS ON THE ELEMENTAL ABUNDANCES IN HALO STARS.**
Justin C. Seymour

116. **USING A PHOTON BEAM FOR THERMAL NOCICEPTIVE THRESHOLD EXPERIMENTS.**
Spencer Sherwood

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2) only papers presented at the annual meeting are eligible for publication,
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F: Proposed Timetable for Manuscript Processing

It is the policy of the Arkansas Academy of Science that 1) at least one of the authors of a paper submitted for publication in the *JOURNAL* must be a member of Arkansas Academy of Science, 2) only papers presented at the annual meeting are eligible for publication, and 3) manuscript submission is due at the annual meeting. Thus, manuscripts should be submitted to the *JOURNAL* website: http://scholarworks.uark.edu/jaas/, two days before the meeting.

After the meeting all correspondence regarding response to reviews etc. should be directed to the Managing Editor. Publication charges ($50 per page) are payable by check (we are unable to accept PO numbers or credit cards) when the corresponding author returns their response to the reviewers' comments. **Publication charges, made payable to the Arkansas Academy of Science, must be sent to the Editor-in-Chief: Dr. Mostafa Hemmati, P.O. Box 1950, Russellville, AR 72811.** Please note that the corresponding author will be responsible for the total publication cost of the paper and will submit one check for the entire remittance by the set deadline. If page charges are not received by the deadline, publication of the manuscript will occur in the following year's *JOURNAL* volume (i.e. two years after the meeting at which the data was presented!) The check **must** contain the manuscript number (assigned at time of submission). All manuscript processing, review and correspondence will be carried out electronically. Thus, authors are requested to add the editors' e-mail addresses to their accepted senders' list to ensure that they receive all correspondence.

Timetable

Please note: All manuscripts must be properly formatted PRIOR to submission as a MS Word document.

All manuscripts must be submitted a minimum of 2 days prior to the annual meeting electronically via: http://scholarworks.uark.edu/jaas/, the *JOURNAL* website. The entire review and publication procedure will be handled via the server. Should you have any problems, please contact the Managing Editor (istill@atu.edu).

End of April: Initial editorial review. Associate Editors are assigned.

End of May: Manuscripts sent to reviewers.

End of July: All reviews received. Editorial decisions made on reviewed manuscripts. Manuscripts returned to authors for response to reviewers' critiques. For accepted manuscripts, additional details and due dates for manuscript return will be given in the acceptance letter. Please email the Managing Editor if you fail to receive your review by the 31st July.

End of August: Authors return revised manuscripts to the *JOURNAL* website, as per due dates in the acceptance letter, typically 28 days after editorial decision/reviewers, critiques were sent. Corresponding author submits publication charges to the Editor-in-Chief (mhemmati@atu.edu): Mailing address: Mostafa Hemmati, P.O. Box 1950, Russellville, AR 72811. The Managing Editor will send an email reminder approximately 1 week prior to the final due date.

The prompt return of revised manuscripts and payment of publication costs is critical for processing of the *JOURNAL* by the *JOURNAL* staff. If the corresponding author will be unable to attend to the manuscript within the framework of this schedule, then it is the responsibility of the corresponding author to make arrangements with a coauthor to handle the manuscript. NB. The corresponding author will be responsible for submitting the total publication cost of the paper by August 31st. FAILURE TO PAY the publication charges by the deadline will prevent processing of the manuscript, and the manuscript will be added to the manuscripts received from the following year's meeting.

PREPARATION OF THE MANUSCRIPT

A. General considerations

Format the manuscript as a published paper. If you are unfamiliar with the *JOURNAL*, please access last year's journal at http://scholarworks.uark.edu/jaas to familiarize yourself with the layout.

1. Use Microsoft Word 2007 or higher for preparation of the document and the file should be saved and uploaded as a Word Document.
2. The text should be single spaced with Top and Bottom margins set at 0.9″ Left and Right margins, 0.6″. Except for the Title section, the manuscript must be submitted in two column format and the distance between columns should be 0.5″. This can be performed in MS Word by clicking on "Layout" on the Toolbar and then "Columns" from the drop-down menu. Then select "two" (columns).
3. Indent paragraphs and subheadings 0.25″.
4. Use 11 point font in Times New Roman for text. Fonts for the rest of the manuscript must be
   a) Title: 14 point, bold, centered, followed by a single 12 point blank line.
   b) Authors' names: 12 point, normal, centered. Single line spaced. Separate last author line from authors' address by a single 10 point blank line.
   c) Authors' addresses: 10 point, italic, centered. Single line spaced. Separate last author line

from corresponding author's email by a single 10 point blank line.
d) Corresponding authors email: 10 point, normal, left alignment.
e) Running title: 10 point, normal, left alignment.
f) Main text: 11 point, justified left and right.
g) Figure captions: 9 point, normal.
h) Table captions: 11 point normal.
i) Section headings: 11 point, bold, flush left on a separate line, then insert an 11 point line space. Section headings are not numbered.
j) Subheadings: 11 point, bold, italic and flush left on a separate line.

6. Set words in italics that are to be printed in italics (e.g., scientific names).

7. In scientific text, **Arabic numerals** should be used in preference to words when the number designates anything that can be counted or measured: 3 hypotheses, 7 samples, 20 milligrams. However, numerals are not used to begin a sentence; spell out the number, reword the sentence, or join it to a previous sentence. Also, 2 numeric expressions should not be placed next to each other in a sentence. The pronoun "one" is always spelled out.

8. A **feature article** is 2 or more pages in length. Most **feature articles** should include the following sections: Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusions, Acknowledgments, and Literature Cited.

9. A **general note** is generally shorter, usually 1 to 2 pages and rarely utilizes subheadings. A note should have the title at the top of the first page with the body of the paper following. Abstracts are not used for general notes.

10. A **review article** should contain a short abstract followed by the body of the paper. The article may be divided into sections if appropriate, and a final summary or concluding paragraph should be included.

Title of a Paper (14 point, bold, centered)

A.E. Firstauthor[1*], B.F. Second[1], C.G. Third[2], and D.H. Lastauthor[1] (12 point font, normal, centered)

[1]Department of Biology, Henderson State University, Arkadelphia, AR 71999
[2]Arkansas Game and Fish Commission, 915 E. Sevier Street, Benton, AR 72015 (10 point font, italic, centered)

[*]Correspondence: Email address of the corresponding author (10 point, normal, left alignment)

Running title: (no more than 65 characters and spaces) (10 point, normal, left alignment)

Figure 1. Layout of the title section for a submitted manuscript.

B. Specific considerations

1. Title section

(see Fig. 1 above for layout).

i. It is important that the title be short, but informative. If specialized acronyms or abbreviations are used, the name/term should be first indicated in full followed by the short form/acronym.

ii. Names of all authors and their complete mailing addresses should be added under the Title. Authors names should be in the form "A.M. Scientist", e.g. I.H. Still. Indicate which author is the corresponding author by an asterisk, and then indicate that author's email address on a separate line (see A.4 for format.)

iii. Please include a Short Informative **Running title** (not to exceed 65 characters and spaces) that the Managing editor can insert in the header of each odd numbered page.

iv. Insert a single 10 point blank line after the "Running Title" and add a Continuous section break.

2. Abstract

An abstract summarizing in concrete terms the methods, findings, and implications discussed in the body of the paper must accompany a **feature article** (or **a review article**). That abstract should be completely self-explanatory. A short summary abstract should also be included for any review article. Please review your title and abstract carefully to make sure they convey your essential points succinctly and clearly.

3. Introduction

An appropriate sized introduction should be included that succinctly sets the background and objectives of the research.

4. Materials and Methods

Sufficient details should be included for readers to repeat the experiment. Where possible reference any standard methods, or methods that have been used in previously published papers. Where kits have been used, methods are not required: include the manufacturer's name and location in brackets e.g. "RNA was prepared using the RNeasy Plus Micro Kit (Qiagen, USA)."

5. Tables and figures (line drawings, graphs, or black and white photographs) should not repeat data contained in the text. Tables, figures, graphs, pictures, etc., have to be inserted into the manuscript with "text wrapping" set as "top and bottom" (not "in line with text"). Figures, tables, graphs and pictures can occupy one column (3.4″ wide) or a maximum of two columns wide (7.3″). In the event that a table, a figure, or a photograph requires larger space than a single column, the two column format should be ended and the Table/figure should be placed immediately afterward. The two column format should continue immediately after the Table/figure. To save space, where possible place Tables/Figures at the top or bottom of the column/page.

Tables and figures must be numbered, and should have titles and legends containing sufficient detail to make them easily understood. Allow two 9 point line spaces above and below figures/tables. Please note that Figure and Table captions should be placed in the body of the manuscript text AND NOT in a text box.

i. Tables: A short caption in 11 point normal should be included. Insert a solid 1.5 point line below the caption and at the bottom of the table. Within tables place a 0.75 point line under table headings or other divisions. Should the table continue to another page, do not place a line at the bottom of the table. On the next page, place the heading again with a 0.75 point line below, then a 1.5 point line at the bottom of the table on the continued page. Tables can be inserted as Tables from Excel, but should not be inserted as pictures from Powerpoint, Photoshop etc., or from a specialized program.

ii. Figures: A short caption should be written under each figure in 9 point, normal. Figure 2 shows an example for the format of a figure inserted into the manuscript. All figures should be created with applications that are capable of preparing high-resolution PhotoShop compatible files. The figure should be appropriately sized and cropped to fit into either one or two columns. Figures should be inserted as JPEG, TIFF images or PhotoShop compatible files. Arrows, scale bars etc., must be integral to the figure: i.e. not "added over" the figure once place in the word document: "independent arrows, etc., will be lost in manuscript formatting. While the *JOURNAL* is printed in black and white, we encourage the inclusion of color figures and photographs that can be viewed in the online version. Please note that the figures directly imported from PowerPoint frequently show poor color, font and resolution issues. Figures generated in Powerpoint should be converted to a high resolution TIFF or JPEG file (see your software user's manual for details).

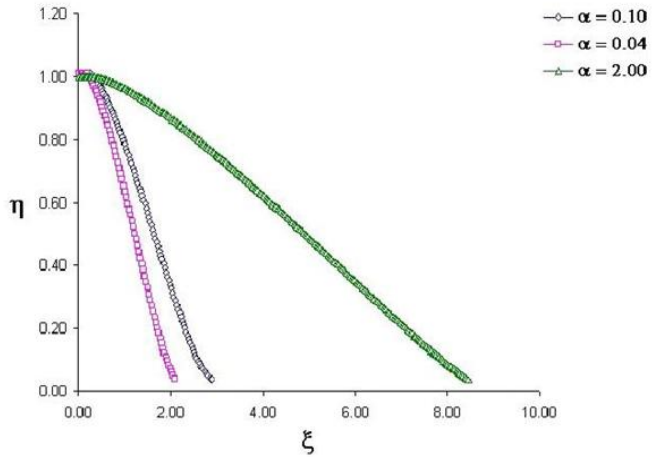

Figure 2. Electric field, η, as a function of position ξ, within the sheath region for three different wave speeds, α.

6. Chemical and mathematical usage

i. The Journal recommends the use of the International System of Units (SI). The **metric system of measurements** and **mass** must be employed. **Grams** and **Kilograms** are units of **mass** not weight. Non-SI distance measurements are permitted in parentheses.

ii. Numerical data should be reported with the number of significant figures that reflects the magnitude of experimental uncertainty.

iii. Chemical equations, structural formulas and

mathematical equations should be placed between successive lines of text. Equation numbers must be in parentheses and placed flush with right-hand margin of the column.

7. Deposition of materials and sequences in publicly available domains

Cataloguing and deposition of biological specimens into collections is expected. Publication of manuscripts will be contingent on a declaration that database accession numbers and/or voucher specimens will be made available to interested researchers. Where possible, collector and voucher number for each specimen should be stated in the Results section. The location of the collection should be stated in the Methods section. This will facilitate easy access should another researcher wish to obtain and examine the specimen in question.

8. Literature Cited

i Authors should use the Name – Year format as illustrated in *The CBE Manual for Authors, Editors, and Publishers* and as shown below. The *JOURNAL* will deviate from the form given in the *CBE Manual* only in regard to placement of authors' initials and abbreviation of journal titles. Initials for second and following authors will continue to be placed before the author's surname. Note that authors' names are in bold, single spacing occurs after periods. If a citation has 9 authors or more, write out the first 7 and append with *et al*. in the Literature Cited section. **Journal titles should be written in full.** Formats for a journal article and a book are shown below along with examples.

ii. Please note how the literature is "cited in text as", i.e. in the introduction, results etc. In general, cite in text by "first author *et al*." followed by publication date. **DO NOT USE NUMBERS, etc.** Also note that in the Literature Cited section, references should be single line spaced, justified with second and following lines indented 0.25". Column break a reference in Literature Cited that runs into the next column so that the entire reference is together. Insert a "Next Page" Section break at the end of the Literature cited section.

Accuracy in referencing current literature is paramount. Authors are encouraged to use a reference databasing system such as Reference Manager or Endnote to enhance accurate citation. Do not cite abstracts and oral, unpublished presentations. Unnecessary referencing of the authors own work is discouraged; where possible the most recent reference should be quoted and appended with "*and references therein*".

General form:

Author(s). Year. Article Title. Journal title volume number(issue number):inclusive pages.

Author(s) [or editor(s)]. Year. Title of Book. Publisher name (Place of publication). Number of pages.

Please note below, that we have included "cited in text as" to show you the form of citation in the text, only.

Specific examples:

Standard Journal Article

Davis DH. 1993. Rhythmic activity in the short-tailed vole, *Microtus*. Journal of Animal Ecology 2:232-8
Cited in text as: (Davis 1993)

Steiner U, JE Klein, and **LJ Fletters.** 1992. Complete wetting from polymer mixtures. Science 258(5080):1122-9.
Cited in text as: (Steiner *et al*. 1992)

Zheng YF and **JYS Luh.** 1989. Optimal load distribution for two industrial robots handling a single object. ASME Journal of Dynamic System, Measurement, and Control 111:232-7.
Cited in text as: (Zheng and Luh 1989)

In press articles

Author(s). Expected publication Year. Article Title. Journal title *in press*.
Cited in text as: (First author *et al. in press*)

Kulawiec M, A Safina, MM Desouki, IH Still, S-I Matsui, A Bakin, and **KK Singh.** 2008. Tumorigenic transformation of human breast epithelial cells induced by mitochondrial DNA depletion. Cancer Biology & Therapy *in press.*
Cited in text as: (Kulawiec *et al. in press*)

Books, Pamphlets, and Brochures

Box GEP, WG Hunter, and **JS Hunter.** 1978. Statistics for experiments. J Wiley (NY). 653 p.
Cited in text as: (Box *et al*. 1978)

Gilman AG, TW Rall, AS Nies, and **P Taylor, eds.** 1990. The pharmacological basis of therapeutics. 8th ed. Pergamon (NY). 1811 p.
Cited in text as: (Gilman *et al*. 1990)

Engelberger JF. 1989. Robotics in Service. MIT Press Cambridge (MA). 65 p.
Cited in text as: (Engelberger 1989)

Book Chapter or Other Part with Separate Title but Same Author(s) – General format is given first.

Author(s) or editor(s). Year. Title of book. Publisher's name (Place of publication). Kind of part and its numeration, title of part; pages of part.

Hebel R and **MW Stromberg**. 1987. Anatomy of the laboratory cat. Williams & Wilkins (Baltimore, MA). Part D, Nervous system; p 55-65.

Singleton S and **BC Bennett**. 1997. Handbook of microbiology. 2nd ed. Emmaus (Rodale, PA). Chapter 5, Engineering plasmids; p 285-96.

Book Chapter or Other Part with Different Authors – General format is given first.

Author(s) of the part. Year. Title of the part. *In:* author(s) or editor(s) of the book. Title of the book. Publisher (Place of publication). Pages of the part.

Weins JA. 1996. Wildlife in patchy environments: Metapopulations, mosaics, and management. *In*: McCullough DR, editor. Metapopulations and wildlife conservation. Island Press (Washington, DC). p 506.

Johnson RC and **RL Smith.** 1985. Evaluation of techniques for assessment of mammal populations in Wisconsin. *In:* Scott Jr NJ, editor. Mammal communities. 2nd ed. Pergamon (NY). p 122-30.

Dissertations and Theses – General format is given first.

Author. Date of degree. Title [type of publication – dissertation or thesis]. Place of institution: name of institution granting the degree. Total number of pages. Availability statement.

The availability statement includes information about where the document can be found or borrowed if the source is not the institution's own library.

Stevens WB. 2004. An ecotoxilogical analysis of stream water in Arkansas [dissertation]. State University (AR): Arkansas State University. 159 p.

Millettt PC. 2003. Computer modeling of the tornado-structure interaction: Investigation of structural loading on a cubic building [MS thesis]. Fayetteville (AR): University of Arkansas. 176 p. Available from: University of Arkansas Microfilms, Little Rock, AR; AAD74-23.

Published Conference Proceedings – General format is given first.

Author(s)/Editor(s). Date of publication. Title of publication or conference. Name of conference (if not given in the 2nd element); inclusive dates of the conference; place of the conference. Place of publication: publisher. Total number of pages.

Vivian VL, ed. 1995. Symposium on Nonhuman Primate Models for AIDS; 1994 June 10-15; San Diego, CA. Sacramento (CA): Grune & Stratton. 216 p.

Scientific and Technical Reports – General format is given first.

Author(s) (Performing organization). Date of publication. Title. Type report and dates of work. Place of publication: publisher or sponsoring organization. Report number. Contract number. Total number of pages. Availability statement if different from publisher or sponsoring organization. (Availability statement may be an internet address for government documents.)

Harris JL and **ME Gordon** (Department of Biological Sciences, University of Mississippi, Oxford MS). 1988. Status survey of *Lampsilis powelli* (Lea, 1852). Final report 1 Aug 86 – 31 Dec 87. Jackson (MS): US Fish and Wildlife Service, Office of Endangered Species. Report nr USFW-OES-88-0228. Contract nr USFW-86-0228. 44+ p.

Electronic Journal Articles and Electronic Books should be cited as standard journal articles and books except add an availability statement and date of accession following the page(s):
Available at: www.usfw.gov/ozarkstreams. Accessed 29 Nov 2004.

Online resources
Citation depends on the requirement of the particular

website. Otherwise use the "electronic journal article" format.

US Geological Survey (USGS). 1979. Drainage areas of streams in Arkansas in the Ouachita River Basin. Open file report. Little Rock (AR): USGS. 87 p. <www.usgs.gov/ouachita> Accessed on 2 Dec 2005.

Cited in text as: (USGS 1979)

Multiple Citations are Cited in text as:

(Harris and Gordon 1988; Steiner *et al*. 1992; Johnson 2006).

8. Submission of Obituaries and *In Memoria*

The Executive Committee and the Journal of the Arkansas Academy of Science welcome the opportunity to pay appropriate professional honor to our departed Academy colleagues who have a significant history of service and support for the Academy and Journal. The editorial staff will consider obituaries for former executive committee members to be included in the Journal. Additional obituaries not meeting these criteria will be forwarded to be posted on the Academy website. We would request that paid up members of the Academy that wish to write an obituary provide a one to two page professional description of the scientist's life that should include details of his/her contribution to the Academy and publication record. The format should follow the two column format and 11pt Times New Roman font. A color or black-and-white photograph to fit in one column should also be provided.

BUSINESS & SUBSCRIPTION INFORMATION

Remittances and orders for subscriptions and for single copies and changes of address should be sent to Dr. Collis Geren, Former Vice Provost of Research & Sponsored Programs and Dean of the Graduate School (Retired), University of Arkansas at Fayetteville, AR 72701, (email: cgeren@uark.edu).

Members may receive 1 copy with their regular membership of $30.00, sustaining membership of $35.00, sponsoring membership of $45.00 or life membership of $500.00. Life membership can be paid in four installments of $125. Institutional members and industrial members receive 2 copies with their membership of $100.00. Library subscription rates from 2009 are $50.00. Copies of most back issues are available. The Secretary should be contacted for prices.


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TABLE OF CONTENTS

FEATURE ARTICLES

GENERAL NOTES